RAT LIVER DNA-DEPENDENT RNA POLYMERASE I IS INHIBITED BY CYCLOHEXIMIDE

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Received July 3,1972

SUMMARY

The DNA-dependent RNA polymerase I of rat liver is specifically inhibited by low concentrations of cycloheximide when prepared using the techniques described. Fifty percent inhibition was observed at 0.15 nanomolar cycloheximide (43 picograms/ml).

INTRODUCTION

Previous reports from this laboratory have shown that both the RNA polymerase I from Blastocladiella emersonii and from Achlya bisexualis were specifically inhibited in vitro by cycloheximide (1,2). Although the enzyme from Blastocladiella was relatively insensitive, that from Achlya was 50% inhibited by 0.45 μM cycloheximide. We have attributed this difference in sensitivity to either actual species differences or to differences in the isolation and assay procedures (2).

A recent report by Higashinakagawa and Muramatsu (3) showed that RNA polymerase I (nucleolar enzyme) obtained from rat liver using the technique of Roeder and Rutter (4) was not appreciably inhibited by cycloheximide in vitro. However, using the techniques described for the isolation of cycloheximide sensitive RNA polymerase I from Achlya (2), we have obtained rat liver polymerase I which is extremely sensitive to the inhibitor.

EXPERIMENTAL PROCEDURE

Isolation of nuclei and preparation of RNA polymerase I Freshly dissected livers from male Long-Evans rats were washed twice in PVP buffer (2) and

cut into 1 cm pieces. The techniques for homogenization, isolation of nuclei, and solubilization and DEAE cellulose chromatography of RNA polymerase were as previously described for Achlya (2).

Assay of RNA polymerase activity RNA polymerase activity was measured in vitro by the incorporation of 8-^{3}H-AMP from 8-^{3}H-ATP into trichloroacetic acid-insoluble product as previously described (2).

RESULTS

Polymerase I was eluted from the DEAE cellulose column at 0.17 - 0.18 M ammonium sulfate. Figure 1 shows that incorporation increased up to an addition of about 1.7 µl of the column fraction containing the majority of the polymerase I activity (1:6 dilution). Further addition of enzyme to the reaction mixture resulted in decreased incorporation in a 1 min assay. In all subsequent experiments, the column fraction was diluted six-fold immediately prior to use and then added to the reaction mixture in a volume of 10 µl.

The incorporation of AMP into RNA by polymerase I increased for 2 min (Figure 2), then stopped. The decrease in acid-precipitable radioactivity after 2 min suggests that the partially purified enzyme preparation was contaminated by ribonuclease.

The RNA polymerase I preparation used is further characterized in Table I. The incorporation of AMP into RNA was dependent on both the addition of DNA and UTP to the reaction mixture. This shows that the activity was in fact DNA-dependent RNA polymerase and not a contamination.

Cycloheximide inhibited rat liver polymerase I at all concentrations tested with 50% inhibition occurring at about 1.5×10^{-10} M inhibitor (43 picograms/ml). Complete inhibition was not obtained at 4.5×10^{-6} M cycloheximide, the highest concentration tested. Cycloheximide inhibition was specific to RNA polymerase I, having no inhibitory effect on polymerase II at 4.5×10^{-6} M. The addition of 10 µl of absolute ethanol to the reaction mixtures had no effect on enzyme activity.

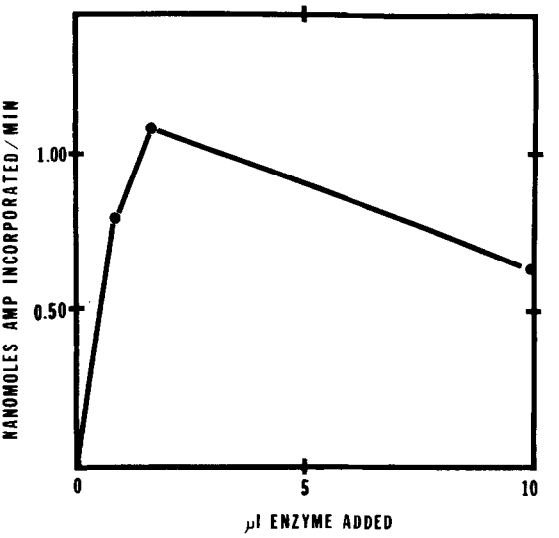

Figure 1. Dilution of RNA polymerase I column fraction. The enzyme fraction from the column was diluted with an appropriate volume of solubilization buffer, and 10 µl was added to each reaction mix. The reaction mix consisted of 0.2 M Tris-Cl, pH 8.1, 3.7 mM $MnCl_2$, 25 mM dithiothreitol, 2.5 mM each of CTP, UTP, and GTP, 2.2 mM ATP, specific activity 6.25 µCi/µmole, 0.25% DOC, 35 µg of T-4 phage DNA in 0.21 ml. The reaction was stopped by the addition of 5 ml of cold trichloroacetic acid. Precipitates were collected on glass fiber filters, washed with 30 ml of trichloroacetic acid and counted in Bray's solution. Assays were for 1 min at 20 C.

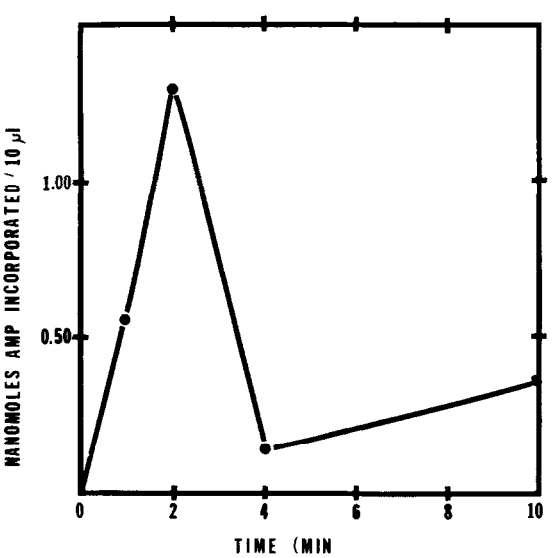

Figure 2. Time course of RNA polymerase I. The column fraction was diluted six-fold and assayed as described in Figure 1 for the times indicated.

TABLE I

Treatment	pmoles AMP incorporated/min
Complete	556
-DNA	0
-UTP	56

UTP and DNA dependency of RNA polymerase I. Undiluted enzyme was assayed as in Figure 1 with the compound listed excluded from the reaction mix.

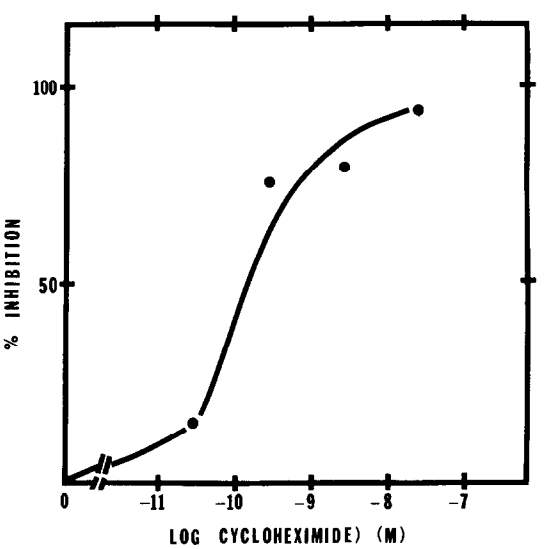

Figure 3. Cycloheximide inhibition of RNA polymerase I. Diluted enzyme was assayed in the presence of cycloheximide at the appropriate concentrations. The inhibitor was added in 10 µl of ethanol, and 10 µl of ethanol were added to all control reactions.

DISCUSSION

The deoxycholate (DOC) technique for the solubilization of RNA polymerase from partially purified nuclei, described by Timberlake et al for use with Achlya bisexualis (2), is effective with nuclei isolated from rat liver. Chromatography of the DOC enzyme from rat liver on a DEAE

cellulose column yields an RNA polymerase I fraction which is very similar to that obtained from Achlya. Rat liver polymerase I obtained using this procedure, unlike enzyme obtained using other procedures, is extremely sensitive to cycloheximide. The distinct difference in sensitivities of Achlya polymerase I and rat liver polymerase I could be due to species differences, or to minor differences in the isolation procedure caused by the difference in tissue. However, the difference between our results and those of Higashinakagawa and Muramatsu (3) indicate that the exact procedure used for the isolation and purification of enzyme is critical to the demonstration of cycloheximide inhibition. The simplest explanation for this phenomenon is that a separable factor is involved in the inhibition, and the differences in techniques result in a differential loss of the factor. A protein affecting ribosomal RNA synthesis in vivo was proposed by Muramatsu et al (5) when they observed that the synthesis of ribosomal RNA in rat liver was specifically inhibited by cycloheximide only after a short lag period. Perhaps the same protein affects sensitivity in vitro.

We wish to thank Mr. Rory Duncan for his gift of the rat livers.

This research was supported in part by grants from the Research Foundation of the State University of New York and the Brown-Hazen Fund of the Research Corporation, Providence, R.I.

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