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CHANGE OF RIBONUCLEASE H ACTIVITY FROM RAT LIVER NUCLEI BY THIOACETAMIDE: PREFERENTIAL INCREASE IN THE ACTIVITY OF MAGNESIUM-DEPENDENT RIBONUCLEASE H

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Summary: The specific activity of Mg^{2+}-dependent ribonuclease H (RNase H) from rat liver nuclei increased three- to four-fold within 24 h of a single injection of thioacetamide, and decreased to normal values 48 h after administration. The response of RNase H activity to thioacetamide was very similar to that of RNA polymerase I after treatment. The activities of Mn^{2+}-dependent RNase H from nuclei and RNase H from soluble fractions were relatively unaffected

Ribonuclease H (RNase H) is an enzyme with the unique ability to degrade RNA strand of RNA-DNA hybrid molecules. It has been isolated from various tissues and cells. The function of this enzym is still uncertain, although it has been suggested that the enzyme might act in DNA replication as well as in transcription (1-7). In order to investigate the possibility that the RNase H might be involved in the control of transcription in rat liver, we studied that behavior of RNase H activity after administration of thioacetamide into a rat. Preceding reports from this laboratory have described the presence of two distinct forms of RNase H in rat liver nuclei, a Mn^{2+}-dependent RNase H of 150,000 daltons and a Mg^{2+}-dependent RNase H of 35,000 daltons, distinguished from one another by sedimentation coefficient, sensitivity to -SH reagents and mode

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of cleavage (8). We now report that the induction of RNA polymerase I activity in rat liver nuclei by thioacetamide is accompanied by a corresponding increase in the activity of nuclear Mg^{2+}-dependent RNase H.

MATERIALS AND METHODS

Unlabelled ribonucleoside triphosphates, calf thymus DNA, bovine serum albumin came from Sigma Chemical Co. 5'-[^{3}H]-UTP (ammonium salt in 50% aqueous ethanol, 23 Ci/mmol) was purchased from Radiochemical Center. *E. coli* RNA polymerase was obtained from Boehringer Mannhiem. Thioacetamide was purchased from Fisher Chemical Co.

Male Wistar rats (100-150 g) were injected intraperitoneally with thioacetamide in 0.15 M NaCl (50 mg/kg body wt). Rats were sacrificed by decapitation. Livers were homogenized with 10 vol of 0.25 M sucrose containing 3.3 mM $CaCl_2$ and centrifuged at 1,000 x *g* for 10 min. The supernatant fluid was then centrifuged at 105,000 x *g* for 60 min to obtain the cytosol fraction. Nuclei were isolated from the particulate fraction of the homogenate by the procedure of Chauveau *et al* (9) using 2.2 M sucrose containing 3.3 mM $MgCl_2$. Nuclei were suspended in 20 vol of 0.15 M NaCl containing 5 mM 2-mercaptoethanol, and stirred with a magnetic stirrer for 20 min at 2°C. The suspension was recentrifuged for 5 min at 10,000 x *g*, the supernatant fluid was collected and assayed for enzymatic activity.

Radioactively labelled DNA-RNA hybrid was synthesized on denatured calf thymus DNA using *E. coli* RNA polymerase as described previously (10).

The assay of RNase H was based on the release, into acid soluble fraction, of radioactive material from [^{3}H]-RNA hybridized to DNA as described previously (5).

RNA polymerase I was measured by its insensibility of high levels of α-amanitin with liver nuclei by the method described previously (11).

Protein was determined by the method of Lowry *et al* (12) and DNA by the method of Burton (13).

RESULTS

The specific activities of RNase H and RNA polymerase I in the nuclear preparations were measured as a function of time after administration of thioacetamide (5 mg/100 g body wt) into a rat. As shown Fig. 1, following a single injection of thioacetamide, enzyme activities of RNA polymerase I had increased several fold at 24 h as described by Anderson *et al* (14). Under these conditions, by using $^{2+}$ as a metal a proportional increase in RNase H activity from nuclear extracts was observed, but when the enzyme assay was performed

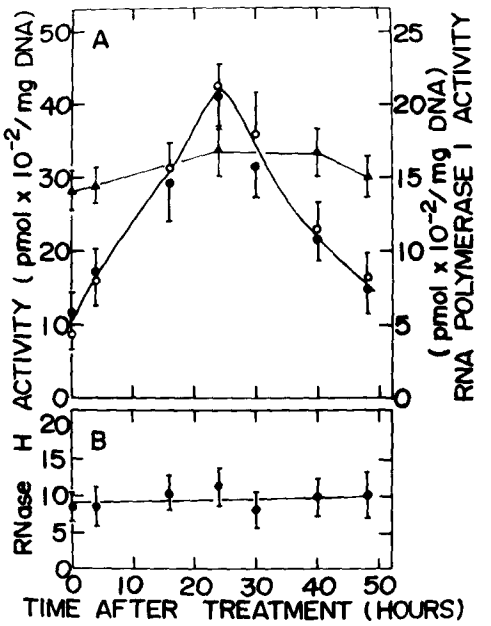

Fig. 1. Effect of thioacetamide administration on the specific activities of RNA polymerase I and RNase H from rat liver. Thioacetamide (50 mg per kg rat wt) was injected at ziro time and liver nuclei and soluble fraction were isolated and assayed as described in MATERIALS AND METHODS. Reaction mixture contained, in a final vol of 0.5 ml, 50 µmol of Tris-HCl (pH 8.0), 5 µmol of $MgCl_2$ or 0.2 µmol of $MnCl_2$, 1 µmol of 2-mercaptoethanol, [^{3}H] RNA-DNA hybrid (5,000 cpm per 150 pmol of ^{3}H-labelled nucleotide) and 50 µl of enzyme solution (about 30 µg of protein in nuclear extract and 0.3 mg of protein in soluble fraction, respectively). After 15 min incubation at 25°C the reaction was stopped by the addition of 0.1 ml of 0.1 M EDTA (pH 7.0), 0.4 mg bovine serum albumin and 0.5 ml of cold 10% trichloroacetic acid. The reaction mixtures were centrifuged and the supernatant was collected and counted in Triton X-100/toluene scintillation fluid. Each point shows the mean ± S.D. from 4-5 rats. A. ○ , RNA polymerase I activity in nuclei; ● , RNase H activity in nuclear extracts with Mg^{2+} as a metal; ▲ , RNase H activity in nuclear extracts with Mn^{2+}. B. ● , RNase H activity in soluble fraction with Mg^{2+}.

using Mn^{2+}, no significant difference in the level of RNase H activity was detected. Twenty-four hours after thioacetamide treatment, the level of both RNA polymerase I and Mg^{2+}-dependent RNase H activities began to decline and reached almost normal level at about 48 h after the injection. When tested for RNase H activity in the soluble

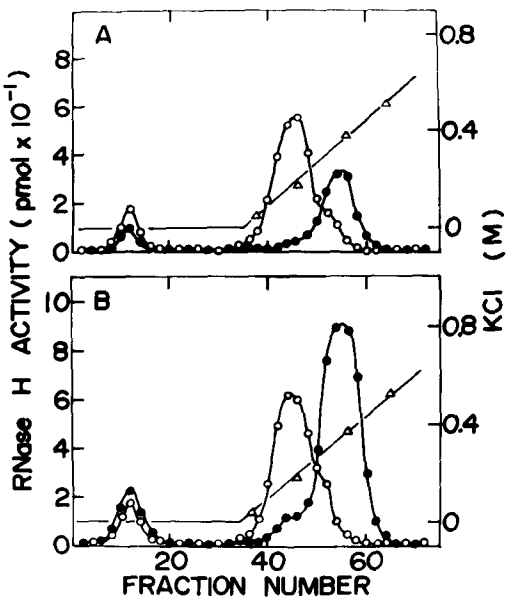

Fig. 2. Effect of a single injection of thioacetamide on the RNase from rat liver nuclei. The nuclei derived from 9 g of liver were suspended in 5 ml of 0.15 M NaCl containing 5 mM 2-mercaptoethanol, and stirred gently with a magnetic stirrer for 20 min at 0°C. The nuclear extracts after centrifugation were dialyzed against 5 mM potassium phosphate buffer (pH 7.7), 5 mM 2-mercaptoethanol and 10% glycerol (Buffer A) for 5 h. The dialyzed nuclear extracts were loaded onto phosphocellulose (P11) columns (6 x 1.6 cm) that had been previously equilibrated with Buffer A. After washing the columns with 2.5 column vol of the same buffer, the elution was carried out with a linear gradient of KCl from 0 to 0.8 M containing Buffer A. Fractions of 1.0 ml were collected and 50 µl aliquots were taken to determine both Mn^{2+}- and Mg^{2+}-dependent RNase H activities. The reaction was studied under same assay conditions described in Fig. 1. The salt concentration was determined by conductance measurements. A. normal rats, B. thioacetamide-treated rats. ○ , Mn^{2+}-dependent RNase H; ● , Mg^{2+}-dependent RNase H; △ , KCl concentration.

fraction by using Mg^{2+} or Mn^{2+}, no enhanced activity of RNase H was observed from zero time to 48 h.

Fractionation of nuclear extracts on a single phosphocellulose column indicated an increase in Mg^{2+}-dependent RNase H activity in carcinogen-treated rats. Fig. 2 shows the phosphocellulose column chromatography profile of nuclear RNase H from normal and thioacetamide-treated rat livers. Two major peaks corresponding to Mn^{2+}-

and Mg^{2+}-dependent RNase H were eluted at 0.16 M and 0.37 M KCl and a minor peak having RNase H activity was recovered in the flow-through from the column. As shown in Fig. 2, the total activity of the Mg^{2+}-dependent RNase H was about 3-fold higher at 24 h after thioacetamide treatment, whereas the activity of Mn^{2+}-dependent RNase H was relatively unaffected with the assay conditions employed.

DISCUSSION

Thioacetamide is a weak carcinogenic agent that increases the nucleolar size and stimulates pre-ribosomal RNA synthesis in liver cells (15). Anderson *et al* (14) have reported that RNA synthetic activity of isolated nucleoli of thioacetamide-treated rat livers increased almost five-fold within 24 h after a single injection of the drug, and the increased activity returned to normal 73 h after injection. Leonard and Jacob (16) have reported that the activity of RNA polymerase IB in the thioacetamide-treated liver was several-fold higher within 24 h of a single injection of the carcinogen, and the effect was relatively specific with respect to the transcriptionally active or chromatin-associated (bound) population of RNA polymerase IB. On the other hand, a stimulatory effect of RNase H on transcription by rat liver RNA polymerase II was reported by Sekeris *et al* (3).

Büsen *et al* (4) have reported that several enzymes with RNase H activity can be identified in bovine lymphoid tissue, and the activity of one of these enzymes (RNase H IIb requires Mg^{2+} for optimal activity increased in parallel with the induction of uridine incorporation after treatment with concanavalin A. The present studies clearly show that thioacetamide can preferentially stimulate the activity of Mg^{2+}-dependent RNase H as well as RNA polymerase I from rat liver nuclei within 24 h after administration. As well documented, RNA

polymerase I, which is present in the nucleolus, is presumably transcribing ribosomal RNA precursors. Recently, an RNase H activity has been found in the most purified preparations of yeast RNA polymerase A (17, 18). The polymerase and nuclease activities were in fact physically inseparable by a number of fractionation techniques. A partially purified RNase H from rat liver nuclei was found to have no RNA polymerase I activity (8). The role of Mg^{2+}-dependent RNase H activity in ribosomal RNA synthesis is still obscure. However, transient DNA-RNA hybrids are formed during transcriptional process in chromatin structure, and Mg^{2+}-dependent RNase H may regulate its template activity including a proof-reading function for the polymerase.

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