Zn^{2+}-INDUCED STIMULATION OF NUCLEAR RNA SYNTHESIS IN RAT LIVER

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1. Introduction

The chemical and structural properties of transition metal-nucleotide complexes and their biochemical implications have been considered [1]. Unfortunately all experiments have failed to isolate a naturally occurring metal-polynucleotide. These metal complexes do not survive the usual extraction procedures where high ionic strength buffers or chelating agents are employed. The only exception was a Hg-DNA complex which appeared to be stable enough to withstand all this treatment [2].

Kinetic studies on the intracellular distribution of $^{65}Zn^{2+}$ revealed that the highest concentration of this metal ion is found in the nuclei [3–5]. Furthermore, Zn^{2+} ions were shown to influence nucleic acid metabolism [6–8]. Intraperitoneal injection of $ZnCl_2$ into rats markedly affected RNA and DNA synthesis in liver nuclei.

In this paper we have attempted to characterize the newly synthesized nuclear RNA induced by Zn^{2+} ions. We have shown that injections of 10^{-4} moles $ZnCl_2$/kg rat stimulate the synthesis of high molecular weight and/or ribosomal RNA. The fractionation pattern of RNA is similar to that obtained with high Mg^{2+} concentrations. The possibility was considered that the activity of Zn^{2+} was due to a secondary effect, namely that it caused an increased secretion of corticoid hormones known to stimulate RNA synthesis [9, 12]. This possibility was excluded by using adrenalectomized rats, in which Zn^{2+} had virtually the same effect as in normal rats.

2. Materials and methods

2.1. *Animals*

Female albino Wistar rats weighing 120 ± 20 g were used throughout. They received food (Altromin R-10) and water *ad libitum*. If necessary total adrenalectomy [13] was performed under narcosis with Evipan (100 mg/kg body weight) 65 hr prior to decapitation. Complete removal of the adrenals was achieved by gently touching the surrounding area with a heated scalpel. The injection of Zn^{2+} was performed intraperitoneally (10^{-4} M $ZnCl_2$/kg rat) 10 hr before the experiment was finished. Liver nuclei were isolated in aqueous sucrose solutions of different densities as described elsewhere [3, 4, 9]. For RNA-labelling *in vivo*, each rat received a 10 min pulse of 2 μCi 6-^{14}C-orotic acid.

2.2. *Chemicals*

^{3}H-UTP (1.5 Ci/mmole), 6-^{14}C-orotic acid (60.8 Ci/mole); the Radiochemical Centre, Amersham. ATP, CTP, UTP, GTP, phosphoenolpyruvate, pyruvate-kinase; Boehringer, Mannheim, Salmon sperm DNA, spermidine.3 HCl; Serva, Heidelberg. Diphenylamine; Merck. HEPES (*N*-2-Hydroxyethylpiperazine-N^1-2-ethylsulphonic acid) A-grade; Calbiochem., Los Angeles, Hyamine; PPO (2,5-diphenyloxazole), POPOP [1,4-di-2-(phenyloxazolyl)-benzene]; Packard. Sucrose, special enzyme grade,

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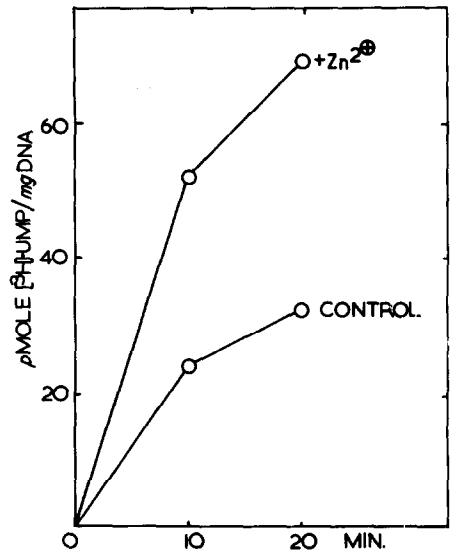

Fig. 1. RNA polymerase activity of liver nuclei from adrenalectomized rats. Each group contained 4 rats. The control group was treated with 0.15 M NaCl. The Zn^{2+} group received a 10 hr Zn^{2+}-pulse (10^{-4} moles $ZnCl_2$/kg rat) intraperitoneally. The assay mixture contained: HEPES, 50 mM, pH 8.4; spermidine. 3 HCl, 5.6 mM; NaF, 8 mM; phosphoenolpyruvate, 2.5 mM; $MgCl_2$, 1.5 mM; GTP, CTP, ATP, 45 μM each; ^{3}H-UTP, 0.45 μM (~1 μCi); pyruvate-kinase (1.5 U); nuclei (= 0.8 μg DNA); incubation volume 0.8 ml, temp. 37°.

lot U 11 49. Mann, N.Y.. All other chemicals were of the purest quality available and obtained through Merck, Flucka or Riedl de Haen.

2.3. *RNA*

RNA was extracted from fresh nuclei employing phenol Na-dodecylsulphate [9, 14]. $E_{280/260}$ ranged between 0.49-0.52. No DNA or protein could be detected. Further fractionation succeeded by applying continuous density gradient centrifugation (5–20% sucrose) in a 25.1 rotor (spinco-L-ultracentrifuge) at 2300 rpm for 16 hr. Fractions were collected in an Ultrorac and subjected to measurements of E_{260} (Zeiss PMQII) or radioactivity (Packard, Tri-Carb; dioxane scintillator [6]). RNA polymerase assay was performed with whole enzymatically active nuclei as already described [6].

3. Results

Specific RNA polymerase activity of isolated enzymatically active whole liver nuclei was determined following a 10 hr pulse of 10^{-4} moles $ZnCl_2$/kg rat. A 65% stimulation of RNA-synthesis compared to the control group could be observed (see also [8]). If this experiment is performed exactly the same way em-

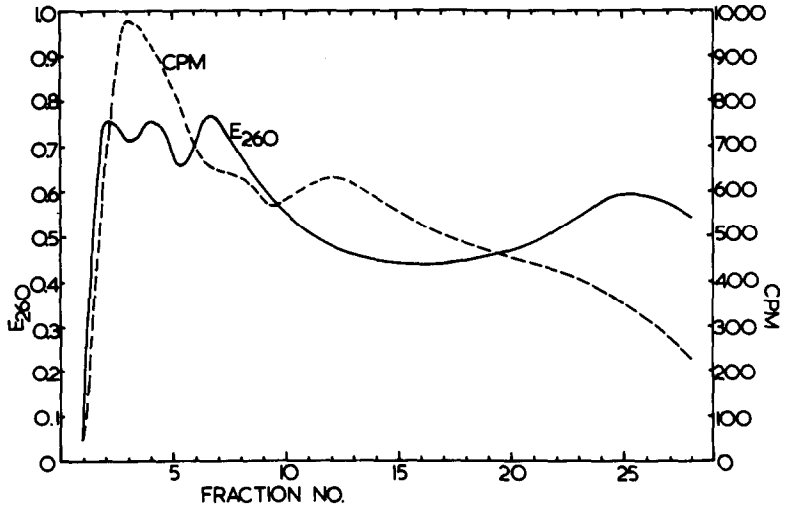

Fig. 2. Elution pattern of a sucrose gradient centrifugation of nuclear RNA after a 10 min pulse of 2 μCi ^{14}C-orotic acid. In addition to sucrose (5–20%) the medium contained 0.01 M NaAc, 0.1 M NaCl, 1 mM EDTA, pH 5.1. Centrifugation was performed in a 25.1 rotor (Spinco-L-Ultracentrifuge) at 23,000 rpm for 16 hr.

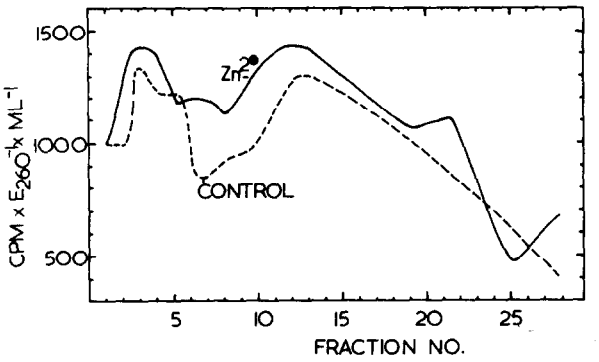

Fig. 3. Incorporation of ^{14}C-orotate into RNA in Zn^{2+}-treated rats and control groups (4 rats per group). $ZnCl_2$ (10^{-4} moles/kg rat) was injected intraperitoneally 10 hr prior to decapitation. Radioactivity is expressed relative to E_{260} in the extracted RNA. cpm $\times$ E_{260}^{-1} $\times$ ml^{-1} of unfractionated RNA was 61% higher compared to RNA from control rats. For further experimental details see fig. 2.

ploying adrenalectomized rats, virtually the same stimulation pattern of Zn^{2+} appears (fig. 1).

In vivo pulse labelled RNA was isolated from rat liver nuclei and subjected to sucrose gradient centrifugation. The elution profile is plotted in fig. 2.

A very similar elution pattern was obtained, when rats previously injected with 10^{-4} M Zn^{2+}/kg were used. However, a drastic increase of high-molecular weight and/or ribosomal RNA can be observed in those rats compared to the control group (fig. 3).

3. Discussion

The removal of the adrenals did not alter the effect of Zn^{2+} ions on RNA biosynthesis. Thus, the increased formation of RNA is not attributable to corticoid hormones. Zn^{2+} Ions at a concentration of 10^{-4} M enhance the synthesis of high molecular weight and/or ribosomal RNA. A similar results was obtained with Mg^{2+} [16] though higher concentrations were needed since the concentration of Mg^{2+} in mammalian organisms is normally much higher than 10^{-4} M. In contrast 10^{-4} Mn^{2+} enhanced the synthesis of low molecular weight RNA [7].

The extraordinarily high uptake of $^{65}Zn^{2+}$ by the nucleus suggests that this cellular compartment is the major site of Zn^{2+} activity [3]. However, we still do not know whether polynucleotide-Zn or protein-Zn complexes, or both, are necessary for polynucleotide synthesis. Further studies on this subject will be awaited with great interest.

Acknowledgement

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