Chemistry of Natural Compounds, Vol. 36, No. 4, 2000

COMPARATIVE INVESTIGATION OF PROTEIN BIOSYNTHESIS IN ANIMAL AND PLANT CELL NUCLEI INFLUENCED BY TZh-85

O. Kh. Saitmuratova

UDC 612.591.04

The effect of TZh-85 (plant growth stimulator) on protein biosynthesis in cell nuclei of hepatocytes and cotton sprouts is studied. This preparation does not stimulate protein synthesis in the nuclei of animal and plant cells.

Key words: growth stimulator, hepatocyte of eukaryote liver nucleus, ^{14}C lysine, cell nuclei.

We have previously demonstrated that certain chemical toxins affect protein synthesis in rhibosomes and cell nuclei of digestive organs [1]. It was found that some of them stimulate protein synthesis whereas others suppress it [1, 2].

The goal of the present work was to reveal differences in the nature of action of TZh-85, a new cotton growth stimulator used in agriculture, on various species of animal and plant eukaryote nuclei.

Aquadiaceto-N-hydroxymethylureazinc hydrate (trivial name TZh-85) is a complex compound of zinc acetate and hydroxymethylurea [3]. We studied in detail the action of this preparation on protein biosynthesis in a model nuclear system of plant cells and nuclei of rat-liver hepatocytes because the liver is the most sensitive to the action of chemical toxins.

Microscopic investigations showed that nuclei isolated from rat-liver hepatocytes and cottonseed sprouts are sufficiently pure and intact. The nuclei of liver hepatocytes are large whereas the nuclei of cotton sprouts are small and round. The rate of protein synthesis was determined from the incorporation of labeled amino acids into synthesized protein. Hepatocyte cell nuclei incorporate the maximum amount of ^{14}C lysine in sodium phosphate buffer at pH 7.4 *in vitro* during incubation for 60 min. Cotton-sprout cell nuclei incorporate the maximum amount of ^{14}C lysine in *TRIS*-buffer during incubation for 40-45 min (Fig. 1).

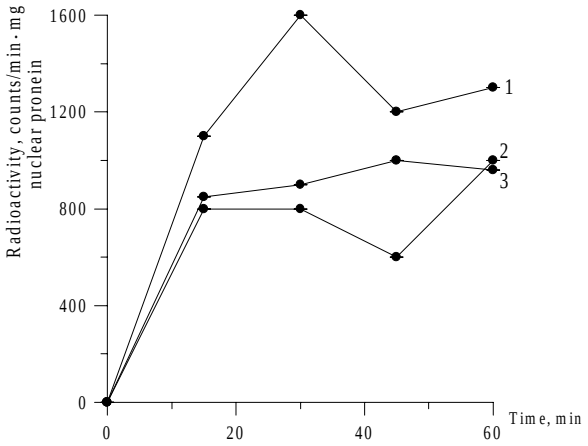

Fig. 1. Kinetics of ^{14}C lysine incorporation into nuclei of synthesized proteins in *in vitro* experiments: nuclei of rat-liver hepatocytes (1), cotton sprouts (2), TZh-85, 3.75 g/kg (3).

A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 162 70 71. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 334-335, July-August, 2000. Original article submitted June 12, 2000.

 0009-3130/00/3604-0408$25.00

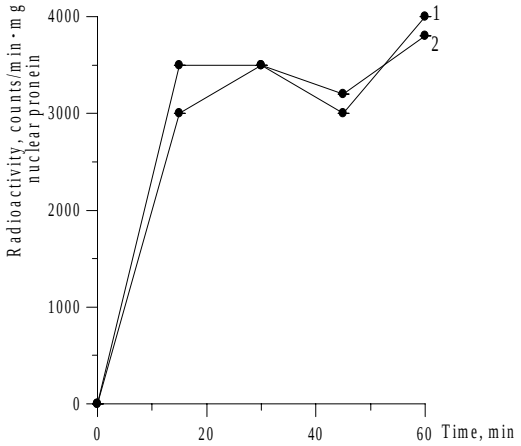

Fig. 2

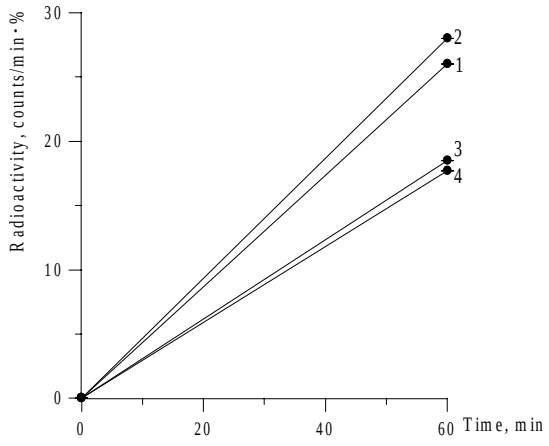

Fig. 3

Fig. 2. Kinetics of protein biosynthesis in cell nuclei of 2-day "Yulduz" cotton sprouts: control (1) and TZh-85 (2).
Fig. 3. Effect of TZh-85 on protein biosynthesis in isolated 2-day nuclei of cotton sprouts: control (1) and TZh-85 at 0.025 (2), 5 (3), and 10 μg/ml (4).

The data show that the kinetics of incorporation of labeled amino acids into animal and plant cell nuclei are identical in nature but not even over time. Liver-hepatocyte nuclei do not function under conditions characteristic of cotton-sprout nuclei.

The influence of TZh-85 on the ability of isolated cell nuclei to incorporate ^{14}C lysine indicated that the preparation does not stimulate protein synthesis in liver-hepatocyte nuclei whereas, on the other hand, it suppresses it by 28% compared with the control (Fig. 1). The effect on plant cell nuclei is almost undetectable (Fig. 2).

It should be noted that hepatocyte nuclei are most sensitive to TZh-85 for three days, after which protein synthesis is gradually restored. Apparently the liver possesses good compensatory and adaptive capabilities and can compensate for the destructive action of TZh-85.

The effect of TZh-85 on cotton sprouts depends on the dose. Thus, a dose of 0.025 μg/ml stimulates insignificantly protein biosynthesis. Doses of 5 and 10 μg/ml suppress the process by 32 and 38%, respectively, compared with the control (Fig. 3). A dose of 0.025 μg/ml slightly stimulates protein biosynthesis in rat-liver hepatocytes. Thus, the stimulating action of TZ-85 on protein biosynthesis in nuclei of animal and plant cells is insignificant.

EXPERIMENTAL

Male white rats of mass 160-180 g and 2-day cottonseed sprouts were used. Nuclei from liver hepatocytes were isolated by the literature method [4]. Protein synthesis was determined from ^{14}C lysine incorporation as described previously [4].

Cottonseed nuclei of the "Yulduz" variety were isolated from 2-day sprouts. Cotton seeds were treated with conc. H_2SO_4 to remove fibers and washed with distilled water until the washings were neutral. Treated seeds were sprouted in rolls of moist filter paper for 2 days at 28°C.

Sprouts were collected and homogenized in solution I (10 mM *TRIS*-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 0.1 mM phenylmethylsulfonyltarate) (PMSF is a protease inhibitor) containing 0.25 M saccharose. The homogenate was filtered through two layers of burlap and centrifuged at 600 rpm for 5 min (K-23). The solid was discarded. The supernatant was centrifuged at 3500-4000 rpm for 15 min. The resulting precipitate of nuclei was suspended in solution I containing 1.8 M saccharose, layered on 1.8 M saccharose in the same buffer in centrifuge tubes (Beckman, SW-27), and centrifuged at 24,000 rpm for 90 min. The precipitate of nuclei was suspended in solution I and centrifuged at 4000 rpm for 15 min. The nuclei purified in this manner were used for further work. All biochemical procedures were performed at 4°C.

Protein synthesis in isolated cottonseed-sprout cell nuclei was studied by ^{14}C lysine incorporation during incubation for 45 min at 37°C on a rocking water bath. For this, nuclei were suspended in an incubation mixture (0.5 ml) containing 0.25 M saccharose, 0.003 M $CaCl_2$, 0.003 M $MgCl_2$, 0.02 M *TRIS*-HCl at pH 7.0 and 0.1 ml of ^{14}C lysine of specific activity

1 μCi/mM. Protein synthesis was stopped by adding trichloroacetic acid. Labeled nuclear proteins were washed as described previously [4]. The purity and integrity of the resulting liver hepatocyte and cottonseed nuclei were checked using an MBI-15 microscope. Protein content was determined by the Lowry method [5]. Smears of the isolated liver and cotton-sprout cell nuclei were prepared, treated with nuclear dyes, and examined under a microscope. The data were analyzed by variational statistics [6]. The TZh-85 preparation was supplied by A. N. Azizov of the Institute of Chemistry of the Academy of Sciences of the Republic of Uzbekistan.

REFERENCES

1. O. Kh. Saitmuratova, E. A. Tursunov, M. A. Ishanbaeva, et al., *Khim. Prir. Soedin.*, 205 (1998).
2. A. M. Rashkes, O. Kh. Saitmruatova, N. K. Khidirova, et al., *Khim. Prir. Soedin.*, 65 (1998).
3. Z. Sh. Khidoyatova, Author's Abstract of a Candidate Dissertation in Biological Sciences, Tashkent (1996).
4. O. Kh. Saitmuratova, F. N. Rustamova, A. A. Sadykov, and V. B. Leont′ev, *Neirokhimiya*, **6**, No. 3, 260 (1987).
5. O. H. Lowry et al., *J. Biol. Chem.*, **193**, 265 (1953).
6. E. V. Montsevichyute and E. V. Eringene, *Patol. Fiziol. Eksp. Ter.*, **8**, No. 4, 71 (1964).