# EFFECT OF THE PESTICIDE FLUOMETURON (COTORAN) ON TEMPLATE RNA SYNTHESIS

**D. Kh. Khamidov, A. K. Mirakhmedov, G. A. Sagatova, and Sh. S. Azimova**

UDC 574.2.24+576.315.42

KEY WORDS: specific binding; template activity; pesticides

Many pesticides are known to interact specifically with intracellular proteins. It has been proved experimentally that the ligand-receptor-mediated mechanism of interaction of xenobiotics with intracellular proteins determines regulation of specific transcription of a particular set of genes also. Xenobiotics, on entering the cell, bind with a specific receptor protein [10]. The ligand-receptor complex, in turn, can bind with increased affinity with particular sites of cellular DNA. Moreover, long term exposure to xenobiotics may lead to amplification and restructuring of specific genes, leading to initiation and to the development of pathology [10].

In this connection the study of the damaging action of the pesticide Cotoran (a urea derivative widely used in agriculture as a herbicide) is of definite interest because the discovery of the principles governing its action of cell metabolism would lead to establishment of the mechanism of the goal-directed detoxication of this class of compound.

The aim of this investigation was to study the specificity of interaction of Cotoran with nuclei and to study its effect on nuclease sensitivity and template activity of rat liver chromatin.

## EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats weighing 120-150 g. The animals were poisoned by intragastric feeding through a tube. The dose of Cotoran was 0.05 $LD_{50}$ (45 mg/100 g body weight). The animals were killed 60 min after poisoning. Chromatin was obtained from the nuclear fraction by the method in [11]. The nuclei thus obtained were suspended in a solution of 10 mM Tris-NaCl, pH 7.9, 0.1 mM $CaCl_2$ to a final concentration of 1 mg DNA/ml. The DNA concentration was measured on an SF-26 spectrophotometer at wavelengths of 260 and 300 nm. The nuclear suspension was preincubated for 5 min at 37°C, after which it was treated with micrococcal nuclear M-I ("Sigma") and DNase I ("Serva"), which were added to the suspension at the rate of 1 $\mu$g DNA/ml in the course of 1 and 60 min. The reaction was stopped by transferring the suspension into an ice bath and adding EDTA solution (pH 7.9) to a final concentration of 2 mM. The nuclear suspension was dialyzed against a buffer solution containing 10 mM Tris-HCl, pH 7.9, 0.2 mM EDTA, as a result of which the nuclei underwent lysis. The solutions thus obtained were centrifuged at 3000*g* for 15 min. The supernatant contained soluble chromatin, which was used for determination of the template activity of the-chromatin in a standard system with RNA-polymerase from *E. coli* ("Sigma") [6]. The level of RNA synthesis was judged from incorporation of $^3$H-UTP into the acid insoluble fraction (specific radioactivity of $^3$H-UTP was 40 MBq). Specific binding of $^3$H-Cotoran with chromatin protein was studied by the method of replacement with an unlabeled analog after administration of labeled Cotoran in vivo [4]. Protein separation was carried out by analytical electrophoresis in 7% polyacrylamide gel [3].

Laboratory of Structural Organization of Biological Membranes, Institute of Biochemistry, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 3, pp. 261-263, March, 1992. Original article submitted June 10, 1991.

 0007-4888/92/0003-0336$12.50 

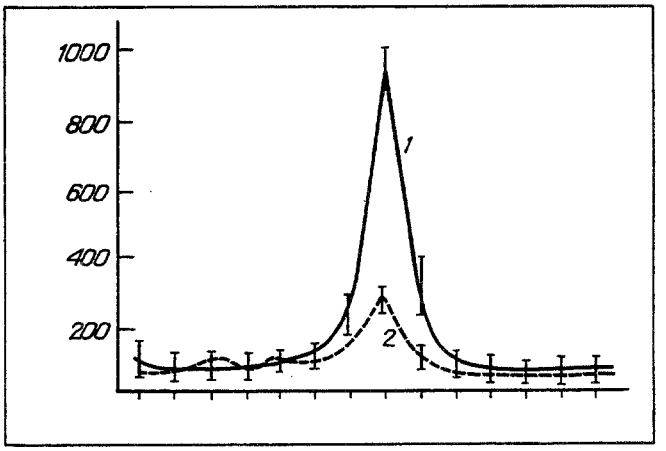


Fig. 1. Distribution of $^3$H-Cotoran in 0.4 M KCl extract of chromatin during cathodal electrophoresis. Curve 1) radioactivity in chromatin proteins during injection of $^3$H-Cotoran into rats, 2) radioactivity in chromatin proteins after their incubation with a 1000-fold excess of unlabeled Cotoran. Abscissa, radioactivity (in cpm); ordinate, length of gel (in cm).

## EXPERIMENTAL RESULTS

Since it was shown previously that $^3$H-Cotoran is mainly located in the nonhistone protein fraction of chromatin [1], we considered it important to study the specificity of interaction of Cotoran with this particular subnuclear fraction. Specific binding of Cotoran with nonhistone proteins of chromatin was studied by the method of replacement with unlabeled analogs after administration of labeled Cotoran to the animals in vivo. For this purpose, the rats received an injection of labeled Cotoran in a dose of 100 $\mu$Ci/100 g, after which the nuclei were isolated and fractionation into subnuclear fractions carried out by the successive extraction method. It was shown that radioactivity in nonhistone proteins of rat liver chromatin was associated with one protein, located in the middle part of the gel (Fig. 1). Consequently, Cotoran binds with a particular chromatin protein. The specificity of its interaction was proved by the fact that a 1000-fold excess of unlabeled Cotoran displaced the label into this particular zone of binding with the above-mentioned protein. Incidentally, other structural analogs of the pesticide Cotoran do not displace the label in the zone of its binding with this protein. The binding constant, measured by Scatchard plot, was $10^{-6}$ M.

It thus follows from our investigations that Cotoran is translocated into the cell nucleus and binds specifically with nonhistone proteins of chromatin.

Similar results were obtained in a study of binding of Cotoran by cytological methods, by studying translocation of labeled Cotoran into interphase nuclei and its acceptance on metaphase chromosomes of cells in culture [5].

There is evidence in the literature that the toxic action of many pesticides on transcription processes is accompanied by a change in sensitivity of certain parts of the chromatin to the action of nucleases [9]. Two of the most frequently used enzymes in research of this kind are micrococcal nuclease and DNase I.

It follows from Table 1 that Cotoran reduces the sensitivity of chromatin to the action of DNase I. For instance, when chromatin is incubated with the enzyme for 60 sec the quantity of DNA liberated from the nuclei is reduced compared with the control by 40%, and after 60 min by 30%.

The sensitivity of the liver chromatin of rats poisoned with Cotoran to the action of micrococcal nuclease also is reduced during the first 60 sec by 30%; later this process stabilizes, and when the enzyme is incubated with chromatin for 60 min the nuclease sensitivity of the liver chromatin of rats poisoned with Cotoran falls by 15% (Table 1).

What events may lead to a change in the sensitivity of chromatin to the action of nucleases? In this case protein–nuclein interaction may be modified. It is this which determines the specific restriction center, which in turn reflects the character of chromatin conformation.

TABLE 1. Sensitivity of Chromatin (in $\mu$g DNA/ml) From Liver of Rats Poisoned with Cotoran, to Action of Nucleases (M ± m)

| Experimental conditions | DNase 1 | | Micrococcal nuclease M-I | |
|---|---|---|---|---|
| | DNA concentration after treatment with nuclease | % | DNA concentration after treatment with nuclease | % |
| 60 sec | | | | |
| Normal | 3931±61 | 100 | 5768±79 | 100 |
| Cotoran | 2404±59 | 60 | 3911±121 | 70 |
| 60 min | | | | |
| Normal | 3165±87 | 100 | 5478±99 | 100 |
| Cotoran | 2118±67 | 70 | 4612±76 | 85 |

TABLE 2. Effect of Cotoran on Template Activity of Chromatin (M ± m)

| Experimental conditions | Template activity of chromatin in presence of RNA-polymerase | | RNA polymerase activity of isolated nuclei | |
|---|---|---|---|---|
| | cpm/μg DNA | % | cpm/μg DNA | % |
| Normal | 3105±100 | 100 | 1935±112 | 100 |
| Cotoran | 2027±92 | 65 | 1114±90 | 60 |

The fact that Cotoran largely reduces the sensitivity of active regions of chromatin to the action of nucleases is evidence of the existence of definite conformational changes in chromatin under the influence of this xenobiotic. The state of sensitivity to nucleases is known to reflect the degree of activity of genes potentially capable or incapable of transcription [12].

In this connection we studied the effect of Cotoran on template activity of rat liver chromatin. We carried out experiments to determine intrinsic RNA-polymerase activity of liver nuclei of rats poisoned with Cotoran. Table 2 shows that intrinsic RNA-polymerase activity of the liver nuclei of rats treated with Cotoran is inhibited by 40% compared with the control. In experiments to study transcription of chromatin in vitro with the aid of RNA polymerase from *E. coli* it was shown that Cotoran induces significant inhibition of template activity of chromatin (by 35%) compared with the control.

It thus follows from the data given above that Cotoran on the whole reduces the template activity of chromatin. It can be concluded that Cotoran evidently disturbs interaction of RNA-polymerase with promoters of genes normally expressed. Considering that in the presence of Cotoran the sensitivity of chromatin to the action of DNase I is reduced, it can be postulated that the Cotoran-binding protein screens the promoter region of chromatin, which is located in the 5′-terminal regulator region of genes normally transcribed.

We know [2] that sensitivity to DNase I is characteristic of a region including at least one active unit of transcription, and it must be expected that under the influence of Cotoran, changes must take place in mRNA synthesis.

It follows from the literature that RNA-polymerase of *E. coli* interacts with the promoter region of DNA containing A-T-rich DNA sequences. Our experiment showed that Cotoran reduces the sensitivity of chromatin not only to the action of DNase I, but also to the action of micrococcal nuclease, which also splits A-T-rich sequences of DNA. Considering that this enzyme possesses definite specificity and interacts with A-T-rich DNA sequences [8], it can be tentatively suggested that a decrease in the number of sites of degradation by micrococcal nuclease in the presence of Cotoran is evidence that competition for the restriction site of the enzymes with Cotoran-binding protein takes place.

Hence it follows from these results that Cotoran binds specifically, in a receptorlike manner, with chromatin proteins. Specific interaction of the pesticide with chromatin proteins probably leads to definite conformational changes in chromatin which, in turn, disturbs template synthesis of mRNA. Repression of certain genes evidently takes place and leads to weakening of the template activity of chromatin under the influence of Cotoran.

LITERATURE CITED

1. Sh. S. Azimova, G. A. Sagatova, D. Khakimova, et al., Uzb. Biol. Zh., No. 4, 7 (1990).
2. B. Lewin, Genes, Wiley, New York (1987).
3. H. Maurer, Disk Electrophoresis. Theory and Practice of Polyacrylamide Gel Electrophoresis [Russian translation], Moscow (1971).
4. V. A. Tkachuk, Introduction to Molecular Endocrinology. Textbook [in Russian], Moscow (1963).
5. D. Kh. Khamidov, A. K. Mirakhmedov, A. A. Arundzhanov, et al., Tsitologiya, **33**, No. 4, 85 (1991).
6. G. Bernardi, Meth. Enzymol., 542 (1977).
7. G. Blobel and V. R. Potter, Science, **154**, 1662 (1966).
8. J. P. Brizik, D. T. Auble, and P. L. De Haseth, Biochemistry (Washington), **26**, No. 3, 950 (1987).
9. R. C. Couch and M. R. Siegel, Pesticid. Biochem. Physiol., **7**, 531 (1977).
10. J. K. Reddy and M. S. Rao, Trends. Pharmacol. Sci., **7**, No. 11, 438 (1986).
11. S. R. Tata and C. C. Widnell, Biochem. J., **98**, 604 (1966).
12. W. J. Wood and C. Felsenfeld, J. Biol. Chem., **257**, No. 13, 7730 (1982).

# CHANGES IN ORGANIZATION OF THE INTERMEDIATE FILAMENT SYSTEM IN HUMAN FIBROBLASTS IN LYSOSOMAL STORAGE DISEASES AND THEIR EXPERIMENTAL MODELS

**T. S. Ivleva, I. S. Tint, A. D. Bershadskii, and G. Ya. Vidershain**

UDC 616-055.5/.7-092:616-008.931:577.152.34]-07

KEY WORDS: lysosomal storage diseases; intermediate filaments

Hereditary insufficiency of one of the acid hydrolases in lysosomal storage diseases (LSD) leads to severe cell damage in various tissues of the body. As a result of progressive accumulation of unsplit compounds of varied nature, hypertrophy of the lysosomal compartment develops, and gradually fills the entire intracellular space. Extensive information has been obtained on the specific molecular biochemical changes lying at the basis of LSD, and is concerned chiefly with different forms of insufficiency of the lysosomal enzymes, the factors necessary for their function in the cell, and the receptor systems responsible for their transport into lysosomes, etc. [14].

However, information on nonspecific intracellular changes in LSD is extremely limited and consists mainly of a description of the morphological features of pathological cells and tissues [13]. Meanwhile, it can be claimed that blocking one stage of metabolism in LSD leads to a number of very serious disturbances in the target cells and, in particular, to changes in the intralysosomal pH [2], to activation of lysosomal enzymes unconnected with the primary defect [1], and others. The study of the cytoskeletal structures in pathological cells is particularly interesting from this standpoint. We know that under normal conditions various organelles, including lysosomes, are bound by some degree to different components of the cytoskeleton, and their dynamic interaction with microtubules (MT) has been studied in most detail [6, 8, 9,

Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the USSR. Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. I. Archakov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 113, No. 3, pp. 263-268, March, 1992. Original article submitted July 3, 1991.

  