ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 53, nr 2: 217-222
1984

Binding of 1-nitro-9- (3-dimethylaminopropylamino)-acridine to the DNA of the apical meristem cells of adventitious onion (*Allium cepa* L.) roots

DANUTA ANTOSIEWICZ

Laboratory of Electron Microscopy, Institute of Botany, University of Warsaw, Krakowskie Przedmieście 26/28, 00-927 Warszawa, Poland

(Received: November 28, 1983. Accepted: January 12, 1984)

Abstract

It was established that one half of the ledakrin (1-nitro-9-(3-dimethylaminopropylamino)-acridine) bound to the DNA in the cells of the studied onion root tips (*Allium cepa* L.), forms labile complexes with it, the remaining half is covalently attached to only one strand of the DNA. One molecule of covalently bound ledakrin falls on average to 10^4-2×10^4 pairs of bases.

Key words: acridine derivative, DNA binding, root tips.

INTRODUCTION

Ledakrin (1-nitro-9-(3-dimethylaminopropylamino)-acridine) is a compound with anticancer properties (Gniazdowski et al. 1979, Radzikowski 1974). In spite of numerous experiments performed with animals and plants, its mechanism of action still remains obscure. It is known that its main mechanism of action in animal cells is a covalent binding to DNA and formation of „cross-links" between two DNA chains. The biologically active form of ledakrin able to form such bonds is its metabolite produced by an enzymatic system which includs cytochrome P-450 (Filipski et al. 1977, Konopa et al. 1976a, b, Krawczyk 1982, Pawlak and Konopa 1979).

In none of the earlier cytological studies was the mutagenic action of this drug noted (Radzikowski et al. 1967, Radzikowski 1974, Wajda 1978). Such an effect was detected when the influence of ledakrin was tested in meristematic cells of onion (*Allium cepa* L.) root tips during the course of mitosis. Chromosome aberrations appeared late

in the postincubation period, attaining a maximum in the 2nd or 3rd 24 h period, that is, about 70-90 h after administration of the drug (Antosiewicz, in preparation).

This study was undertaken to establish if and how ledakrin combines with the DNA of apical meristem cells of onion adventitious roots.

MATERIAL AND METHODS

Roots of onion bulb (*Allium cepa* L.) were grown at room temperature (19-22°C) in dark vessels (one bulb per one vessel) containing deionised water and were constantly aerated. Water was changed every 24 h. When the roots reached a langeth of 2.5-3.5 cm, the onions with their roots were transferred for 24 h to a radioactive 0.1 ppm ledakrin solution in deionised water. The ledakrin was labelled with ^3H in the ring (synthesised at the Technical University of Gdańsk) spec. act. 11.94 $Ci \cdot mol^{-1}$. After incubation, all of the apical meristems (2-3 mm) were cut off and used for isolation of DNA. Two experimental series were run together with controls.

DNA was isolated according to the modified method of Marmur (1961). The apical meristems (8 g) frozen in liquid nitrogen were ground with quartz sand, next 80 cm^3 of a warm (50°-60°C) 0.15 M NaCl, 0.1 M EDTA, 2.5% SDS, pH 8-8.5 solution were added and grinding was continued. After filtration through double cheese cloth, 5 M $NaClO_4$ was added to a final concentration of 1 M. Deproteinization was achieved by using an equal volume of a mixture of chloroform-isoamyl alcohol (v:v, 19:1). After centrifugation the water phase was collected, DNA precipitated with a double volume of cold 96% ethanol and wound onto a glass rod. After drying, the DNA was dissolved in 0.1 SSC and the solution concentration adjusted to 1×SSC. DNA was then repeatedly deproteinized until protein did not appear at the interphase. The water phase was then collected and the DNA was ethanol-precipitated and wound onto a glass rod. After dissolving, the DNA was digested by RNAse (500 $\mu g \cdot cm^{-3}$, bovine pancrease, Sigma) for 2 h at 37°C. Next 5 M NaCl was added to a 1 M concentration and digestion with pronase (500 $\mu g \cdot cm^{-3}$, Calbiochem, 45.000 P.U.K./g) at 37°C was carried out for 2 h. Deproteinization was performed until the disappearance of proteins at the interphase. The DNA was again precipitated with ethanol and wound on a glass rod, dried, dissolved in 0.1×SSC and adjusted to 1×SSC. This DNA solution was used for further analyses.

DNA concentration was determined spectrophotometrically at 280 nm. The absorption coefficient of a solution of 50 μg DNA $\cdot cm^{-3}$ was accepted as 1.00 A. The degree of deproteinization of DNA was determined as the absorption ratio at 260/280 nm.

The amount of ledakrin incorporated into DNA and the type of its binding were determined from measurements of the radioactivity of the native DNA solution and of the supernatants obtained after centrifugation of the DNA precipitated after thermic and acidic (10 M $HClO_4$) denaturation. Ledakrin covalently bound to DNA remains in the sediment after denaturation. Radioactivity was measured by liquid scintilation in a Beckman LS-900 counter for 10 min. To each 100-mm^3 sample, 0.5 cm^3 soluene and 5 cm^3 of a Bray's scintillator (Bray 1960) was added. The measurements were repeated three times.

In order to check whether ledakrin combines with one or two DNA chains, denaturation and renaturation of DNA was investigated by measuring extinction at 260 nm with constant warming of the solution from 30°C to 90°C and then cooling to the starting temperature. The whole process was run automatically. The rate of temperature rise was $1.8°C \cdot min^{-1}$ and of cooling $5°C \cdot min^{-1}$. The temperature changes were recorded using a Beckman 7M Programmer. Extinction was measured with an ACTA M VI spectrophotometer, the melting temperature curves were recorded with a Hewlett Packard 7015 A x-y recorder.

RESULTS AND DISCUSSION

DNA isolated from the cells of onion root tips was of high purity. This was indicated by the extinction ratio of 260/280 nm, amounting in all cases to more than 1.7.

The estimation of radioactivity of the purified DNA solutions showed that ^3H-ledakrin was incorporated into DNA (Table 1). For the determination of the nature of this incorporation, the DNA was submitted to acidic denaturation. This was done by precipitation of DNA from the solution with 10 M $HClO_4$ and consequent centrifugation. It appeared that under these conditions an average of 50% of the radioactivity was released into the supernatant (Table 1). This represents the quantity of the ^3H-ledakrin bound noncovalently with DNA. Similar results were obtained after thermal denaturation. In comparison, the amount of ledakrin forming labile complexes with rat DNA corresponds to about 60-70% of the activity of the DNA solution before its denaturation (Krawczyk 1982). Thus, the presented results are close to those obtained for animal cells.

The remaining 50% of radioactivity appears in the DNA sediment. This suggests that this proportion of ^3H-ledakrin is covalently bound to DNA. One molecule of ledakrin is covalently bound to a DNA fragment consisting of, on the average, 10^4-2×10^4 base pars. The amount of covalently bound ledakrin in mice and rats was, for example six molecules

Table 1

Distribution of radioactivity in the solution of native DNA, in the supernatant and in the DNA sediment obtained after acidic (10 M $HClO_4$) denaturation

No. of experiment	DNA solution in 1×SSC			Supernatant	DNA sediment	
	concentration	radioactivity		radioactivity		^{3}H-ledakrin amount, %
	$\mu g \cdot cm^{-3}$	cpm · 100 mm^{-3}	cpm · μg^{-1} DNA	cpm · 100 mm^{-3}	cpm · μg^{-1} DNA	
I	0.8	680	8500	329	4387	52
II	0.9	362	4022	189	1922	48

per 10^3 base pairs (Konopa et al. 1976b, Filipski et al. 1977). The binding rate of ledakrin with DNA in onion cells is thus, somewhat lower than in animal cells. Ledakrin also binds covalently to DNA *in vitro* after activation with thiol compounds. In experiments with calf thymus cells, the value of 7 molecules per 10^4 base pairs was obtained (Ślaska et al. 1979).

Changes in the extinction of control DNA and ledakrin-treated DNA tested during thermal denaturation and renaturation are presented in Figs. 1a and b. As it can be seen, the presence of covalently bound ledakrin does not change the rate of renaturation. This indicates that ledakrin is covalently bound with only one DNA strand.

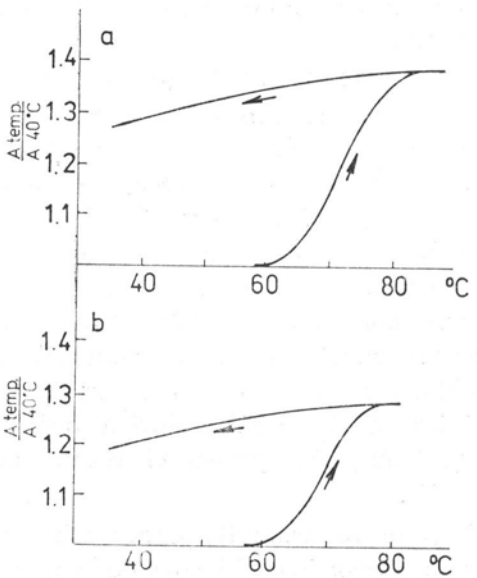

Fig. 1. Thermal denaturation and renaturation profiles of DNA from apical meristems of onion roots; a — after administering ledakrin in 0.1 ppm concentration for 24 h; b — control. Arrows indicate the direction of changes of the temperature

Intercalation of acridines with DAN occurs spontaneously, whereas formation of covalent bonds requires previous transformation of acridines into derivatives capable of reacting with DNA. The metabolic transformation of the acridine derivative, ledakrin, into a compound capable of forming covalent bonds with DNA has so far only been ascertained in animal cells. The demonstration of the formation of such bonds with DNA of meristematic onion root cells gives evidence that this compound can be metabolized by plant cells also. The absence of DNA cross-links in plant cells after ledakrin administration indicates a somewhat different metabolic pathway than in animal cells. Metabolism of various chemical compounds to active mutagens in plants has been noted earlier. Metabolic activation of sodium azide has been described in barley embryos (O w a i s et al. 1978) and of 1.2-dibromoethane in maize (S c o t t et al. 1978). These experiments indicate that the metabolic pathway of 1.2-dibromoethane in the microsomal system of mammals and plant extracts is similar. Numerous insecticides and pesticides are also metabolised in plants and the mode of this metabolism in animals and plants is frequently similar (M e n n and S t i l l 1977).

Similarities in the molecular mechanism of action of ledakrin in animal and plant cells has been demonstrated in the present paper. Results showing the mutagenic activity of ledakrin in onion meristematic cells will be published elsewhere (Antosiewicz, in preparation). Therefore, further investigations with particular attention paid to the effect of ledakrin after a long time following its administration seem to be very promising.

Acknowledgments

The author wishes to thank Dr. W. J a c h y m c z y k from the Department of Genetics of the Institute of Biochemistry and Biophysics at Warsaw, and Dr. Z. K r a w c z y k from the Department of Tumor Biology of the Institute of Oncology in Gliwice for help in the experiments and much valuable advice in the course of this work.

REFERENCES

B r a y G. A., 1960. A simple assicient liquid simpillator for counting aqueaus solutions in a liquid scintillation counter. Analyt. Bioch. 1: 279-285.

F i l i p s k i J., M a r c z y ń s k i B., S a d z i ń s k a L., C h a l u p k a G., C h o r ą ż y M., 1977. Interactions of some nitro-derivatives of substituted 9-aminoacridine with DNA. Bioch. Biophys. Acta 478: 33-43.

G n i a z d o w s k i M., F i l i p s k i J., C h o r ą ż y M., 1979. Nitracine: In: Antibiotics V/2. F. E. Hahn (ed.). Springer-Verlag, Berlin-Heidelberg-New York, pp. 275-297.

Konopa J., Chotkowska E., Kołdej K., Matuszkiewicz A., Pawlak J. W., Woynarowski J. M., 1976a. Studies on the mechanism of antitumor activity of ledakrin. Mat. Med. Pol. 8: 258-265.

Konopa J., Kołdej K., Pawlak J., 1976b. Covalent binding of 1-nitro-9(3--dimethyl-n-propylamino)-acridine, a new antitumor drug, to DNA of Ehrlich ascites tumor cells *in vivo*. Chem. Biol. Interact. 13: 99-103.

Krawczyk Z., 1982. Inhibition of RNA-polymerase B activity in normal and regenerating rat liver after administration of 9-aminoacridine. Acta Biochim. Pol. 29: 259-273.

Marmur J., 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. 3: 208-218.

Menn J. J., Still G. G., 1977. Metabolism of insecticides and herbicides in higher plants. CRC Crit. Rev. Toxic. 1: 1-21.

Owais W. M., Zarowitz M. A., Gunovich R. A., Hedgdon A. L., Kleinhofs A., Nilan R. A., 1978. A mutagenic *in vivo* metabolite of sodium azide. Mutat. Res. 53: 355-358.

Pawlak J. W., Konopa J., 1979. *In vitro* binding of metabolically activated (^{14}C)-ledakrin, or 1-nitro-9-^{14}C-(3'-dimethylamino-n-propylamino)-acridine, a new antitumor and DNA cross-linking agent, to macromolecules of subcellular fractions isolated from rat liver and HeLa cells. Bioch. Pharm. 28: 3391-3402.

Radzikowski C., 1974. Preparat C-283. Zjednoczenie Przemysłu Farmaceutycznego „Polfa" Warszawa.

Radzikowski C., Więckowska Z., Woźniakowska Z., Chorąży M., 1967. The cytotoxic and cytostatic effect of the derivative of 1-nitro-9-aminoacridine in tissue culture. Neoplasma 14: 121-134.

Scott B. R., Sparrow A. H., Schwommer S. S., Schairer L. A., 1978. Plant metabolic activation of 1,2-dibromoethane (EDB) to a mutagen of greater potency. Mutat. Res. 49: 203-212.

Ślaska K., Szmigiero L., Jaros-Kamińska B., Ciesielska E., Gniazdowski M., 1979. The mechanism of inhibition of DNA transcription *in vitro* by nitracine (ledakrin, C-283). Molec. Pharm. 16: 287-296.

Wajda L., 1978. Wpływ pochodnej akrydyny (1-nitro-9(3-dwumetyloaminopropyloamino)-akrydyny×2 HCl) na mitozę w korzeniach cebuli (*Allium cepa* L.). In: Mutageneza roślin wyższych. Uniwersytet Śląski, Katowice, pp. 218-223.

Wiązanie 1-nitro-9-(3-dwumetyloaminopropyloamino)-akrydyny z DNA komórek merystemów wierzchołkowych korzeni przybyszowych cebuli (Allium cepa L.)

Streszczenie

W pracy badano wiązanie się ledakrinu (1-nitro-9-(3-dwumetyloaminopropyloamino)-akrydyny) z DNA komórek merystemów wierzchołkowych korzeni przybyszowych cebuli (*Allium cepa* L.). Po 24-godzinnej inkubacji z ^{3}H-ledakrinem stwierdzono, że połowa tego związku połączonego z DNA tworzy z nim labilne kompleksy, natomiast pozostałe 50% łączy się kowalencyjnie z jedną z nici DNA. Jedna cząsteczka ledakrinu przypada na 10^4-2×10^4 par zasad.