A C T A  U N I V E R S I T A T I S  L O D Z I E N S I S
FOLIA BIOCHIMICA ET BIOPHYSICA 6, 1988

Ewa Ciesielska, Mariola Piestrzeniewicz, Władysław Gałasiński,
Marek Gniazdowski

# PROPERTIES OF NITRACRINE-NUCLEIC ACIDS COMPLEXES IN SELECTED ENZYMATIC SYSTEMS*

Some properties of the covalent complexes of the anticancer drug - nitracrine (1-nitro-9-(3,3-N,N-dimethylaminopropylamino) acridine, Ledakrin, C-283, NAC) with nucleic acids have been tested in DNA or RNA--dependent enzymatic systems.

NAC-DNA complexes exhibit decreased template activity in an RNA synthesis in vitro system. They show an increased resistance to DN-ase I and DN-ase $S_1$ and to acid hydrolysis. Binding of NAC to tRNA decreases aminoacylation of the latter. On the other hand, such aminoacyl--tRNA does not loose activity in the protein synthesis in vitro. Preliminary experiments on the chromatographic resolution of the hydrolyzed complexes are presented here.

An anticancer drug, nitracrine (1-nitro-9-(3,3-N,N-dimethylamino-propylamino) acridine, NAC, Ledakrin, C-283) inhibits DNA, RNA, and to a lower extent protein synthesis either in vivo or in the cell culture (see [4] for review). In the presence of sulfhydryl compounds several biologically active 1-nitroacridine derivatives form covalent complexes with DNA, RNA and proteins. A structure-activity relationship exhibited by the nitroacridines both in this reaction and in the biological experiments prompted us to use the thiol-dependent system as a model of the drug fate in the cell. It has been found that complexes of NAC with DNA show considerably lower template activity in the RNA synthesis in vitro system [5, 6, 12, 13]. A sensitivity of NAC-DNA complexes to nucleases and

* This work was supported by Medical Academy of Łódź under the project No. 14.

the effect of the drug binding to tRNA on tRNA function were assayed in the experiments reported here. They enabled a further characterization of the NAC-DNA complexes and approaching other possible molecular mechanisms of the drug action.

## MATERIALS AND METHODS

9[$^{14}$C] NAC, the unlabelled drug and synthetic polynucleotides were the same as described before [3, 11]. Yeast tRNA isolated according to [15], pancreatic deoxyribonuclease I (E.C. 3.1.21.1). DNA (Worthington, USA), deoxyribonuclease $S_1$ from Aspergillus oryzae (E.C. 3.1.30.1, Calbiochem, USA) were used. Other analytical grade chemicals were purchased from Cefarm, Poland.

Complexes of NAC with DNA, tRNA or synthetic polynucleotides were formed in the presence of dithiothreitol, purified and their stoichiometry was estimated as described before [11].

Aminoacylation of tRNA was assayed according to Mans and Novelli [8] using yeast tRNA as a control and an equivalent amount of NAC-tRNA complex, U-[$^{14}$C] amino acid mixture (Amersham England) and rat liver aminoacyl-tRNA synthetases preparation (E.C. 6.1.1). Translation test was performed as described before [7].

DN-ase I digestion assay was carried out in 50 mM Tris/HCl buffer pH 7.5 containing 4 mM $mg^{2+}$, 1 unit of the enzyme and 30 µg of DNA or an equivalent amount of the NAC-DNA complex per ml. The complexes were formed as described above with DNA previously purified by phenol extraction. The samples were incubated at 20°C and the course of the reaction was followed for two hours by the absorbance measurements at 260 nm.

DN-ase $S_1$ digestion assay was performed according to V o g t [14] as described by P u l k r a b e k [10] using 0.28 mg of the control DNA or the NAC-DNA complex and 0.002 I.U. of the enzyme per ml.

Paper chromatography of the [$^{14}$C] NAC - polynucleotide complexes after hydrolysis (0.3 M HCl at 100°C for 1 h) was performed in the isopropanol (concentrated HCl) $H_2O$ solvent (65 : 16.7 : 18.3). The samples corresponding to 200-300 µg of neutralyzed hydrolyzate were spotted onto Whatman paper No. 2 and developed for 18 h by the descending technique. Then radioactivity of 2 cm paper chromatogram strips was measured in liquid scintillation counter using toluene scintilator with 0.4% PPO and 0.01% POPOP.

## RESULTS AND DISCUSSION

Effect of NAC on tRNA function. In order to check whether irreversible binding of NAC to tRNA imparis its function, NAC-tRNA complex containing on average two drug molecules per RNA chain was formed in the presence of dithiothreitol and assayed for aminoacyl accepting activity and its ability to maintain translation process. When the drug-tRNA complex was used in aminoacyl-tRNA synthesis system the amount of radioactive amino acids bound to tRNA was 58% of the control (Tab. 1).

Table 1

Aminoacylation of the NAC-tRNA complex

Aminoacylacja tRNA związanego z NAC

| Specification | CPM[a] | % control |
|---|---|---|
| tRNA control (20 µg) | 638 ± 72 | 100 |
| NAC-tRNA (20 µg) | 373 ± 52 | 58 ± 8 |
| tRNA control (10 µg) | 472 ± 22 | 74 ± 3 |
| NAC-tRNA (10 µg) | | |

[a] CPM = counts per minute.

On the other hand, when [$^{14}$C] aminoacyl-tRNA preformed either with the control or NAC-treated tRNA were added to the protein synthesis in vitro system no differences in the amino acid incorporation to the acid insoluble material were observed (not shown). Hence the drug inhibits amino acid binding to tRNA to some extent while it does not seem to affect the further steps of protein synthesis. However the inhibition of aminoacylation is relatively low when compared to the drug effect on the transcriptional template activity of DNA. RNA synthesis on DNA bearing comparable amount of NAC molecules covalently bound (i.e. 20-30 drug molecules per $10^3$ of nucleotides) is reduced to few percent [11, 13]. A relative insensitivity of aminoacylation may be due to the NAC preference to guanyl residues shown either with synthetic polyribonucleotides or polydeoxynucleotides [3]. There are some guanyl moieties in the helical part of amino acid accepting arm which is probably responsible for binding of tRNA-synthetase [1, 9]. Aminoacyl residues are however coupled to CCA sequences which obviously do not contain guanine. A low effect of the drug on the tRNA

functions presented here and a high inhibition of RNA synthesis in vitro [5, 12, 13] is in good agreement with the observations made in vivo indicating that the drug inhibits RNA synthesis to a higher extent than protein synthesis [2].

Sensitivity of NAC-DNA complex to nucleases. Binding of NAC to DNA decreases both the rate and the extent of hydrolysis of the complex with pancreatic DN-ase I (Fig. 1) and DN-ase $S_1$ from Aspergillus oryzae (Fig. 2).

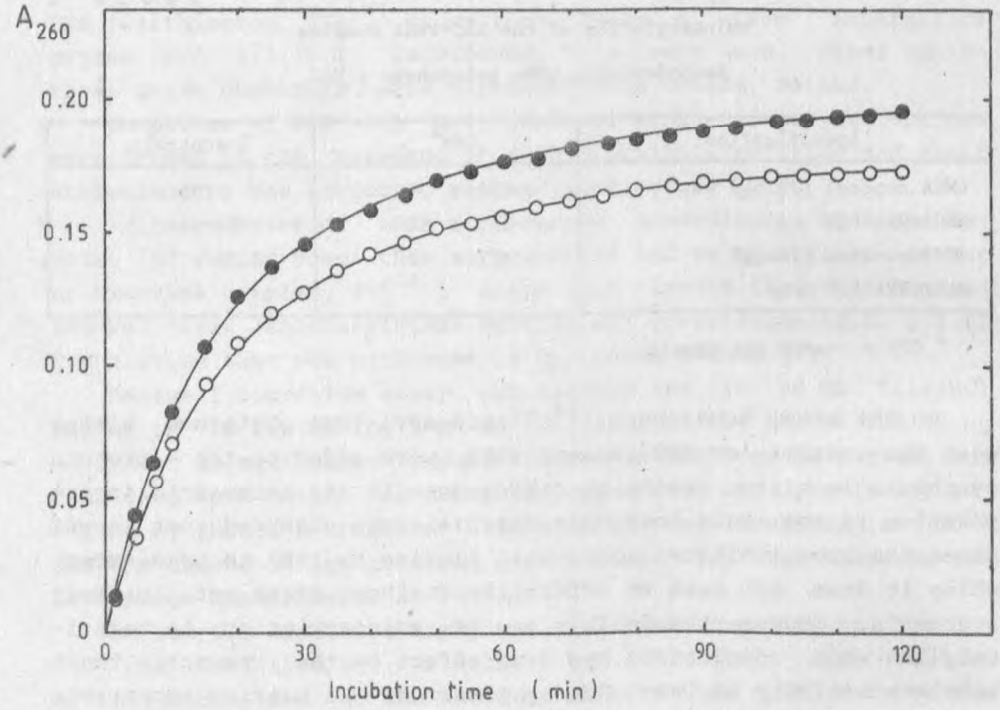


Fig. 1. Time course of DNA and NAC-DNA digestion by DN-ase I
●—● control DNA (1), ○—○ NAC-DNA complex (2)

Rys. 1. Trawienie DNA wolnego i związanego z NAC przez DN-azę I
1 - DNA kontrolne, 2 - DNA związane z NAC

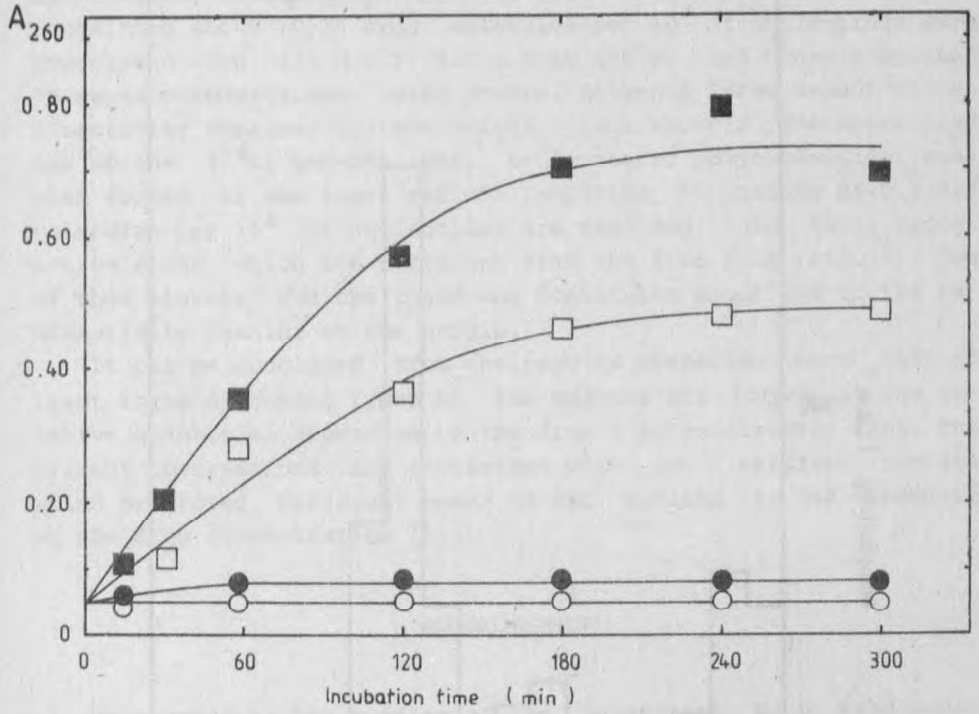


Fig. 2. Time course of DNA and NAC-DNA digestion by DN-ase $S_1$

●—● control native DNA (1), ■—■ control heat-denatured DNA (2), ○—○ NAC--DNA complex (3), □—□ heat-denatured NAC-DNA complex (4)

Rys. 2. Trawienie DNA wolnego i związanego z NAC przez DN-azę $S_1$

1 - natywne DNA kontrolne, 2 - kontrolne DNA denaturowane termicznie, 3 - DNA związane z NAC, 4 - DNA związany z NAC denaturowany termicznie

Lower sensitivity of the complex (either non-heated or after thermal denaturation) to the latter enzyme is of interest. DN-ase $S_1$ shows a specificity to single-stranded DNA. Low affinity of the enzyme to native DNA in the complex indicates that no single--strand regions appear in DNA upon the drug binding. The time course curve of the reaction of denatured NAC-DNA complex with DN-ase $S_1$ falls well below the corresponding curve obtained with the heated control DNA (Fig. 2). This result is consistent with our previous observations [3] that interstrand links are introdu-

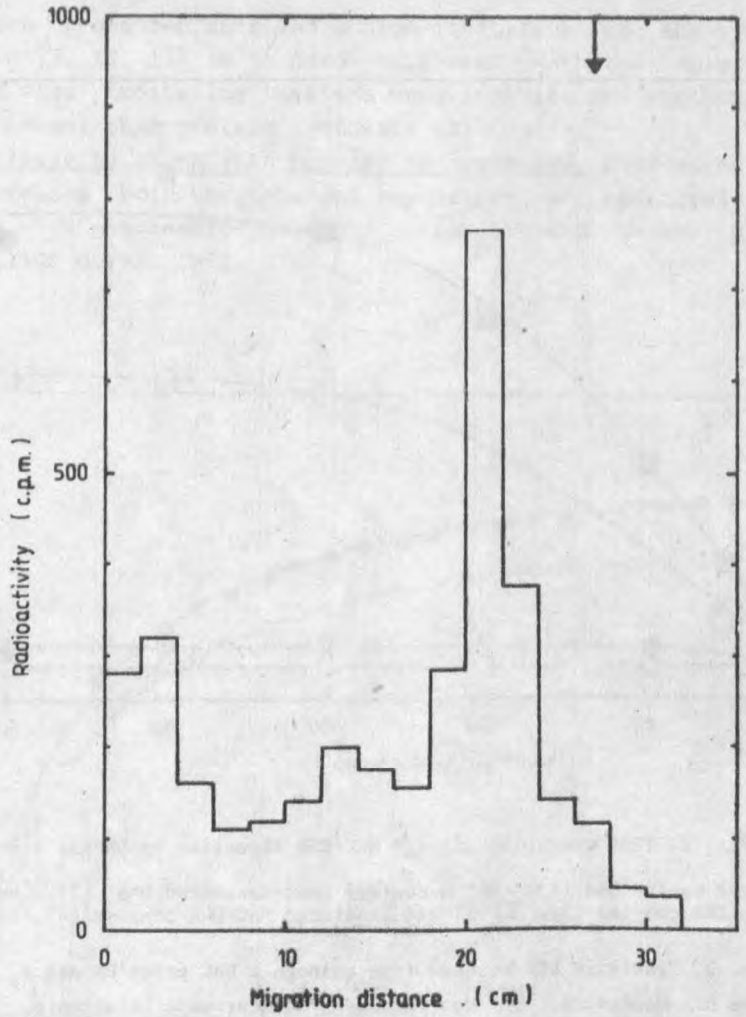


Fig. 3. Paper chromatography of [$^{14}$C] NAC-RNA complex after its acid hydrolysis

Rys. 3. Chromatografia bibułowa produktów chemicznej hydrolizy RNA związanego z [$^{14}$C] NAC

ced to DNA upon the adduct formation. The existence of the cross--links in the complex enables a partial reconstitution of the double-stranded structures in heat denatured DNA hence decreasing its sensitivity to the single-stranded specific nuclease $S_1$.

Chemical hydrolysis of NAC-polynucleotide complexes. Complexes of NAC with DNA or RNA exhibit relatively high resistance in va-

rious conditions particularly when formed at the high drug - polynucleotide molar ratio. When [$^{14}$C] NAC-DNA or RNA complexes containing about 10-20 drug molecules per $10^3$ of nucleotides were hydrolyzed with 0.3 M HCl for 1 h at 100°C, and then subjected to paper chromatography with several solvents large amount of radioactivity remained at the origin (not shown). The hydrolyzates of the [$^{14}$C] NAC-DNA, RNA, or synthetic polynucleotide complex formed at the lower rations resulting in binding of 0.2-0.8 molecules per $10^3$ of nucleotides are resolved into three radioactive spots which are different from the free drug (Fig. 3). Two of them migrate and the third one containing about 20% of the radioactivity remains at the origin.

It can be concluded from the results presented here that at least three different types of the adducts are formed at the relative quantities depending on the drug - polynucleotide ratio. The present observations are consistent with our earlier results which predicted different modes of NAC binding to DNA depending on the drug concentration [11].

ACKNOWLEDGEMENT

We appreciate the reading of the manuscript by E. Szadowska. M.A, and the capable technical assistance of Mrs M. Frasunek.

REFERENCES

[1] Chambers R. W. (1971), Progr. Nucl. Acid Res. Molec. Biol., 11, 33.

[2] Filipski J., Marczyński B., Chorąży M. (1975), Acta Biochim. Pol., 22, 119.

[3] Gniazdowski M., Ciesielska E., Szmigiero L. (1981), Chem.-Biol. Interaction, 34, 355.

[4] Gniazdowski M., Filipski J., Chorąży M. (1979), Antibiotics. Mechanism of action of antieukaryotic and antiviral compounds, ed. F. E. Hahn, Springer-Verlag, Berlin, Vol. V/2, 275.

[5] Gniazdowski M., Szmigiero L., Śląska K. Jaros-Kamińska B., Ciesielska E. (1975), Mol. Pharmacol., 11, 310.

[6] Gniazdowski M., Śląska K., Szmigiero L., Jaros-Kamińska B. (1972), Stud. Biophys., 31/32, 417.
[7] Jabłonowska K., Telejko E., Kopacz-Jodczyk T., Gałasiński W. (1981), Bull. Acad. Pol. Biol. Cl. II, 29, No. 9-10, 361.
[8] Mans R. J., Novelli G. D. (1961), Arch. Biochem. Biophys., 94, 48.
[9] Mirzabekov A. D., Lastity D., Levina E. S., Bayev A. A. (1971), Nature New Biol., 229, 21.
[10] Pulkrabek P., Leffler S., Weintein I. B., Grunberger D. (1977), Biochemistry, 16, No. 14, 3127.
[11] Szmigiero L., Gniazdowski M. (1981), Arzneim.-Forsch. Drug Res., 31, 1875.
[12] Szmigiero L., Śląska K., Ciesielska E., Jaros-Kamińska B., Gniazdowski M. (1977), Acta Biochim. Polon., 24, 35.
[13] Śląska K., Szmigiero L., Jaros-Kamińska B., Ciesielska E., Gniazdowski M. (1979), Mol. Pharmacol., 16, 287.
[14] Vogt V. M. (1973), Eur. J. Biochem., 33, 192.
[15] Wasiak T., Gniazdowski M. (1978), FEBS Letters, 89(2), 260.

Department of General Chemistry
Institute of Physiology and Biochemistry
Medical Academy of Łódź

Department of General and Organic Chemistry
Institute of Chemistry
Medical Academy of Białystok

Ewa Ciesielska, Mariola Piestrzeniewicz, Władysław Gałasiński,
Marek Gniazdowski

## WŁAŚCIWOŚCI KOMPLEKSÓW NITRAKRYNY Z KWASAMI NUKLEINOWYMI W WYBRANYCH UKŁADACH ENZYMATYCZNYCH

Przebadano niektóre właściwości kompleksów leku przeciwnowotworowego, nitrakryny (1-nitro-9-(3,3-N,N-dimetyloaminopropyloamino) akrydyna, Ledakrin, C--283, NAC) z kwasami nukleinowymi w wybranych układach enzymatycznych zależ-

nych od DNA lub RNA. Kompleksy NAC-DNA charakteryzują się obniżoną aktywnością matrycową w układzie syntezy RNA in vitro. Wykazują one pewną oporność na działanie DN-azy I i DN-azy $S_1$ i na hydrolizę chemiczną. Wiązanie NAC do tRNA obniża jego aminoacylację. Z drugiej strony, taki aminoacylo-tRNA wykazuje niezmienioną aktywność w układzie syntezy białka in vitro. W niniejszym artykule przedstawiono również wstępne wyniki chromatografii zhydrolizowanych kompleksów.