# DNA-TROPIC INHIBITORS OF NUCLEASE ACTION ON DNA IN LIVER NUCLEI

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The deoxyribonucleases (DNases) perform widely different functions in cell activity. They take part in DNA replication in vivo, in repair processes, in DNA catabolism, and also in injury to DNA arising in certain pathological processes accompanied by liberation of DNases from lysosomes. The DNases undoubtedly play a role in growth processes and in division of tumor cells. The ability of certain DNA-tropic agents, used in the treatment of cancer and of certain parasitic diseases, to inhibit the action of DNases has been described.

The writers previously obtained data on the inhibition of degradation of free DNA through the action of DNase I by certain DNA-tropic agents.

The object of this investigation was to study the effect of these agents on DNase I activity in the nuclei and also on the activity of certain nuclear nucleases, taking into account the great complexity of this system: DNA is degraded while in the composition of nuclei, where it is partly bound with proteins, in consequence of which the kinetics of the process is more complex than that of degradation of free DNA.

## EXPERIMENTAL METHODS

Preparations of calf thymus DNA (from "Calbiochem"), DNase I (from "Serva"), and the following DNA-tropic agents were used: distamycin A and chromomycin $A_3$ (from "Boehringer Mannheim GmbH"), actinomycin D (from "Reanal"), carminomycin and bleomycin (from the Institute for the Search for New Antibiotics, Academy of Medical Sciences of the USSR), ethidium bromide (from "Calbiochem"), and proflavine (from "Sigma"). Activity of the DNase I preparations was verified spectrophotometrically [7].

Nuclei were isolated from the liver of albino rats weighing 150-200 g in isotonic sucrose solution, using four treatments with 0.5% Triton X-100 [5].

To estimate the action of $Mg^{++}$-activated nuclease, an incubation medium containing 0.01 M NaCl, 0.03 M $MgCl_2$, and 0.01 M Tris-HCl, pH 7.4, was used [8]. The nuclear suspension, containing 300-400 μg/ml DNA was incubated in the presence of one of the DNA-tropic agents at 37°C for 180 min. Degradation of intranuclear DNA in the absence of the agent served as the control. To prevent bacterial growth in the samples sodium azide was added to a final concentration of 0.02%. Aliquots were taken from the reaction mixture in the course of incubation and mixed with cold $HClO_4$ solution to a final concentration of 7%, and then centrifuged at 28,000g for 20 min. Nucleic acids were determined in the supernatant from the difference in absorption at 270 and 290 nm [4].

To assess the action of Ca, Mg-dependent endonuclease an incubation medium of the following composition was used: 60 mM KCl, 15 mM NaCl, 0.15 mM spermine, 0.5 mM spermidine, 15 mM 2-mercaptoethanol, 0.34 M sucrose, 1 mM EDTA, 0.2 mM EGTA, 1 mM $CaCl_2$, 10 mM $MgCl_2$, and 15 mM Tris-HCl, pH 7.4 [6]. Nuclease action was estimated from the yield of hydrolysis fragments in 10 mM Tris-HCl, pH 8.0, as described previously [2]. Incubation was carried out at 37°C for 40 min. The DNA concentration in the suspension of nuclei was about 1 mg/ml. DNA was isolated from fractions of soluble deoxyribonucleoprotein (DNP) and the insoluble residue by dodecylsulfate-phenol deproteinization by the method described in [1]. Electrophoretic fractionation of DNA was carried out in 2.5% polyacrylamide gel in glass tubes measuring 5× 100 mm. About 60 μg DNA was introduced into each tube. A current of 5 mA was applied to the tube for electrophoresis. The gels were stained with 0.01% toluidine blue solution in 1% acetic acid in electrode buffer.

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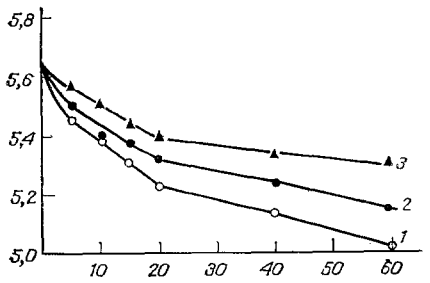


Fig. 1

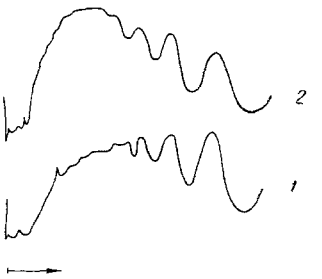


Fig. 2

Fig. 1. Dependence of logarithm of concentration of intranuclear DNA, not hydrolyzed to acid-soluble DNA, on duration of action of DNase I. 1) In absence of agent; 2) in presence of 40 μg/ml (156 nmoles/ml) proflavine; 3) in presence of 40 μg/ml (76 nmoles/ml) distamycin. Abscissa, time (in min); ordinate, ln [S].

Fig. 2. Densitograms of gels after electrophoretic fractionation of DNA fractions soluble in 10 mM Tris-HCl, pH 8.0, obtained by cleavage of nuclear chromatin by Ca, Mg-dependent endonuclease. 1) DNA of soluble intranuclear DNP incubated in the absence of DNA-tropic agents; 2) DNA of soluble intranuclear DNP incubated in presence of 20 μg/ml (38 nmoles/ml) distamycin.

TABLE 1. Action of Certain DNA-Tropic Agents on Hydrolysis of Free and Intranuclear DNA by DNase I

| Agent | Concentration | | $K_e/K_c$ | | |
|---|---|---|---|---|---|
| | μg/ml | nmoles/ml | free DNA | intranuclear DNA | |
| | | | | phase I | phase II |
| Distamycin A | 40—44 | 76—84 | 0,30—0,36 | 0,66* | 0,50* |
| | 100 | 190 | 0,07 | 0,05 | 0,25 |
| Proflavine | 40 | 156 | 0,35 | 0,93 | 0,83 |
| Ethidium bromide | 40 | 100 | 0,33 | 1,00 | 1,00 |
| Actinomycin D | 40 | 32 | 0,83 | 1,00 | 1,00 |

Legend: 1) $K_e/K_c$ – ratio of velocity constant of reaction in presence of agent to constants in control. 2) * – Median of seven determinations.

TABLE 2. Comparison of Concentrations of DNA-Tropic Agents (in nmoles/ml) Causing Equal Inhibition of DNase I Hydrolysis of Free and Intranuclear DNA

| Agent | Intranuclear DNA | Free DNA |
|---|---|---|
| Distamycin A | 76 | 57 |
| | 190 | 110 |
| Proflavine | 156 | 22 |

TABLE 3. Action of Some DNA-Tropic Agents on Hydrolysis of Intranuclear DNA by $Mg^{++}$-Activated Nuclease

| Agent (40 μg/ml) | Concentration, nmoles/ml | Inhibition of reaction, % during incubation for 180 min |
|---|---|---|
| Bleomycin | 29 | 0 |
| Carminomycin | 78 | 21 |
| Actinomycin D | 32 | 43 |
| Chromomycin $A_3$ | 32 | 61 |
| Proflavine | 156 | 80 |
| Ethidium bromide | 100 | 100 |
| Distamycin A | 76 | 100 |

## EXPERIMENTAL RESULTS

A previous investigation showed that degradation of DNA in nuclei by DNase I is a rather more complex process than degradation of free DNA. DNA in liver nuclei is hydrolyzed by DNase I much more slowly (it takes 60 min) than free DNA. To achieve a comparable reaction velocity, in the first case an enzyme concentration about 40 times higher (1.8 Kunitz unit) is required than in the second case (0.045 Kunitz unit). A characteristic feature of degradation of intranuclear DNA is the existence and combination of two phases which differ particularly sharply in velocity: fast and slow hydrolysis. These may be called I and II respectively. Regions of dependence of ln [S] on t ([S] denotes the concentration of unhydrolyzed substrate, t the reaction time), corresponding to these phases, are characterized by a definite angle of slope, the tangent of which expresses the velocity constant of the reaction (Fig. 1).

When the action of DNA-tropic inhibitors in the nucleus is studied what is in fact observed is the combined action of the agents tested with chromatin proteins. It will be clear from Fig. 1 that in the presence of DNA-tropic agents (in this case proflavine and distamycin) inhibition of nucleolysis is observed. Under these circumstances the selected agents had no qualitative effect on the kinetics of the process and, for that reason, the ratio of the velocity constant of DNA hydrolysis in the presence of the agents to the velocity constant in the control can serve as the primary characteristic of the change in velocity of nucleolysis.

Tables 1 and 2 summarize the results of a comparative study of the action of certain DNA-tropic agents on hydrolysis of free and intranuclear DNA by DNase I. Considering the unequal number of functional groups, their different role in interaction with DNA, and the considerable differences in the molecular weights of the agents tested, preference must be given on a priori grounds only to concentrations expressed in terms of weight or only in terms of molar units.

Inhibitors of DNase I such as ethidium bromide, proflavine, and distamycin, which are similar in their effectiveness with respect to hydrolysis of free DNA (Table 1), differed sharply in their ability to inhibit the action of the enzyme on intranuclear DNA. The effectiveness of the first two was considerably lower in the nuclei, and only distamycin showed a significant inhibitory action in concentrations of the same order as during its action on free DNA (Table 2). Probably most combining sites of the less effective agents on DNA are covered by chromatin proteins, and only distamycin, which binds along the small groove [3], manifested its action fully. Actinomycin D occupied an intermediate position as regards effectiveness on free and intranuclear DNA. Carminomycin and bleomycin were ineffective inhibitors of the action of DNase I on both free DNA and intranuclear DNA.

The ratio between the effectiveness of the different inhibitors was different in the case of their action on Mg-dependent intranuclear nuclease (Table 3): Actinomycin D, ethidium bromide, and proflavine were approximately equal in their effectiveness in this case to distamycin and chromomycin. The combining sites of intranuclear DNases and of these inhibitors on chromatin DNA are evidently similar and are not covered by chromatin proteins.

The results of the experiments to study the effect of distamycin on the action of Ca, Mg-dependent nuclear endonuclease did not contradict qualitatively the picture observed with the $Mg^{++}$-activated nuclease. Distamycin in a concentration as low as 20 μg/ml (38 nmoles/ml) significantly inhibited the reaction when the DNA concentration in the nuclear suspension was 1 mg/ml (i.e., when the distamycin : DNA ratio was 1 : 50): The yield of the fraction soluble in 10 mM Tris-HCl, pH 8.0, was 61% in the control and 43% in the experiment with distamycin.

Electrophoretic analysis of soluble chromatin DNA fractions showed that DNA of soluble intranuclear DNP, treated with distamycin, was hydrolyzed by Ca, Mg-dependent endonuclease to a much lesser degree than DNA from the control nuclei (Fig. 2). This is clear from the proportion of fractions possessing the highest electrophoretic mobility: It is smaller in the first case.

It can thus be concluded from the results of this investigation that distamycin is the most universal inhibitor of hydrolysis of both free and intranuclear DNA by DNase I and by intranuclear endonucleases. Its advantage over intercalating agents is particularly clearly marked in the case of inhibition of the action of intranuclear DNase I.

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# PARTIAL PURIFICATION AND SOME PROPERTIES OF γ-GLUTAMYL TRANSFERASE FROM RAT LIVER AND HEPATOMA


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γ-Glutamyl transferase (GGT) is a membrane-bound enzyme which catalyzes the transfer of the γ-glutamyl radical from glutathione and other γ-glutamyl compounds to amino acids and oligopeptides and also, to a much lesser degree, catalyzes the hydrolysis of these compounds. High GGT activity is found in the mammalian kidney where, it is considered, the enzyme is responsible for transport of certain amino acids through the cell membrane [4, 5]. The functional role of GGT in the liver has not been explained, but there are grounds for considering that it is evidently considerably wider in the liver than in the kidneys [1]. A sharp increase in specific activity of GGT has been demonstrated in hepatomas by comparison with the normal liver [1, 2, 6, 7], but no comparative studies of the enzyme from normal liver and hepatoma for the same species of mammals have hitherto been undertaken.

The object of this investigation was to study partially purified preparations of GGT from rat liver and from transplantable G-27 rat hepatoma.

## EXPERIMENTAL METHODS

Preparations of washed membranes were obtained by the method described previously [1]. GGT activity was determined in the reaction between 5 mM L-γ-glutamyl-p-nitroanilide (from Calbiochem, U.S.A.) and 50 mM glycyl-glycine (from Reanal, Hungary) at pH 8.1; the reaction velocity was determined by measuring the increase in optical density of liberated p-nitroaniline at 405 nm.

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