Effect of Pancreatic Lipase on Supramolecular DNA Complexes in Eukaryotic Cells *In Vivo* and *In Situ*

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Effect of pancreatic lipase on supramolecular DNA complexes, nuclear matrix, and 70% ethanol-fixed cells is studied by elastoviscosimetry. It is shown that lipase induces DNA degradation not only *in vitro* but also in whole cells. Possible role of neutral lipids, in particular, DNA-bound diglycerides, in the arrangement of chromosomal DNA is discussed.

Key Words: *supramolecular DNA complexes; nucleotides; eukaryotic cells, lipase, degradation*

It has been previously shown that supramolecular DNA complexes (DNA-SC) isolated from various eukaryotic cells by mild phenol extraction apart from RNA and non-histone protein (1-2%) contain 0.12-1.2% (depending on cell type) DNA-bound lipids (neutral lipids dominate over phospholipids), which can be extracted by the method of Folch only by treating these complexes with DNase I [9,10]. Various experimental approaches (sedimentation, rotation viscosimetry, electron microscopy, and light scattering) have demonstrated [5,10,11] the resistance of DNA-SC to proteolytic enzymes and their susceptibility for lipophilic agents (Triton X-100, deoxycholate, 35% ethanol) and phospholipases C, A, and D, which induced degradation of the complex into fragments with a molecular weight of about 10^8 D.

Pancreatic lipase produces smaller degradation products ($40\text{-}50\times10^6$ D), which can be attributed to the presence of considerable amount of diglycerides (30% of neutral lipids in the complex [10]), a substrate of this enzyme.

In the present study we demonstrate degradation of DNA-SC by lipase not only in solution, but also in whole cells and in nuclear matrix and discuss a possible role of neutral lipids (diglycerides) in the organization of chromosomal DNA.

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MATERIALS AND METHODS

Using the method of phenol extraction we isolated two DNA fractions, water-soluble DNA-SC and water-insoluble DNA of phenol nuclear matrix (DNA-PNM) concentrated in the phenol—water interface, from various eukaryotic cells (rat thymus, erythrocytes, loach sperm, sarcoma-37, Zeidel ascitic hepatoma, leukemia L1210 and P388 cell lines). Isolation of DNA-SC and DNA-PNM as well as capillary elastoviscosimetry and rotation viscosimetry were described in details in our previous reports [10,11]. Effects of exogenous enzymes on whole cells were studied in cell suspension (2×10^7 cells/ml, initial concentration) fixed with 70% ethanol using standard cytofluorometric technique. In brief, 100 μl-aliquot of ethanol-treated cell suspension was centrifuged (2 min, 3000*g*), the pellet was resuspended in 100 μl Hanks' solution, and 0.5% diethyl pyrocarbonate was added for inhibition of endogenous nucleases. The cell and DNA-PNM was lysed with a mixture containing 1 M NaCl, 0.05 M EDTA, and 0.25% Triton X-100, pH 8) at 24°C for 1 h (DNA-PNM) or 24 h (cells) as described previously [2,3,12]. Lipase from porcine pancreas (Serva) preincubated with phenylmethylsulfonyl fluoride (inhibition of proteolytic activity was experimentally verified), RNase-A (Merck) incubated at 80°C for 15 min, pronase P (Serva), bacterial endonuclease, phospholipase C from *Bacillus cereus* (Calbiochem), lipoperoxide-free olive oil,

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and DNA from chick erythrocytes (Hungary) were used in the experiment. The data are representative of 3-5 independent experiments. Significance of the differences was evaluated using the Student's *t* test.

RESULTS

DNA-SC from rat thymus rapidly degraded in the presence of pancreatic lipase (Fig. 1). Competitive inhibitors of lipase (olive oil) and bacterial endonuclease (commercial DNA) specifically inhibited these enzymes. This allows one to attribute DNA-SC degradation to hydrolysis of its lipase-susceptible bonds, rather than to contamination of the preparation with endogenous nucleases.

Elastoviscosimetric titration of DNA-SC from loach sperm with ethidium bromide (EB) revealed DNA loops (data not shown), which is consistent with our previous data [10,11]. The absence of a two-phase response to a rise of EB concentration attests to the absence of supercoiled DNA. In accordance with previous data [5,10] lipase treatment results in separation of DNA loops and a decrease in DNA molecular weight to 40×10^6 D.

We have previously demonstrated [10] the presence of DNA-bound lipids in DNA-PNM from sarcoma 37 and Zeidel ascitic hepatoma. Similarly to DNA-SC, in DNA-PNM neutral lipids prevailed over phospholipids. Moreover, diglycerides constitute about 30% of neutral lipids. Elastoviscosity titration analysis showed (Table 1) that lipase completely separates loops in DNA-PNM and cleaves it in much the same way as it does with DNA-SC. Incubation of nuclear matrix from leukemia P338 cells with different lipases (pancreatic and *Rhisopus sp.*) modifies its protein spectrum and reduces ^{3}H-thymidine and ^{14}C-sodium acetate incorporation [3], while phospholipase C has no effect on the protein spectrum of nuclear matrix [1,3]. On the other hand, treatment with phospholipase C [14] and sphingomyelinase [1] leads to a considerable (by 80%) but not complete elimination of newly synthesized DNA from the nuclear matrix of rat liver, neutral lipids (unfortunately, not identified in this study) being the only component remained. Thus, two types of contacts between DNA loops and nuclear matrix can be deduced: stable (through neutral lipids) and dynamic (functional, through phospholipids).

This effect of lipase was also observed in whole cells. As seen from Table 2, incubation of different cells with lipase dramatically decreased elastoviscosity of nucleoid DNA (by 90%), similar effect was obtained with DNA-PNM (Table 1). It should be noted that in whole cells 0.005% M EDTA (complete inhibition of bacterial endonuclease) had no effect of

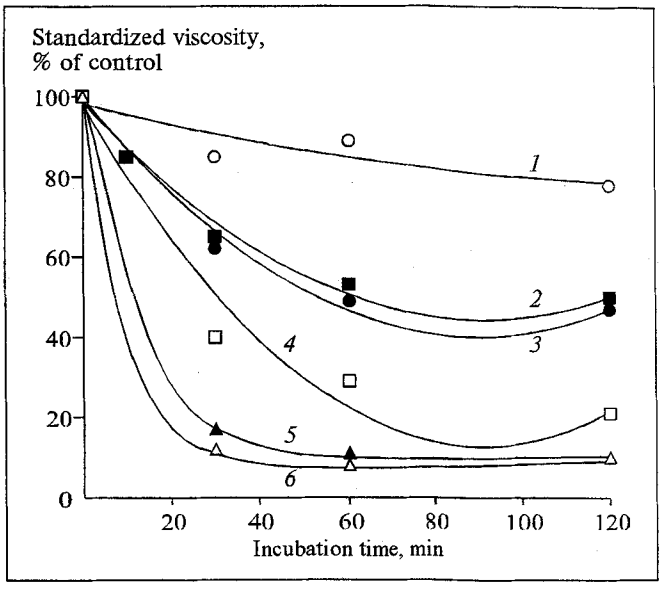

Fig. 1. Effect of pancreatic lipase and bacterial endonuclease on elastoviscosity of supramolecular DNA complexes from rat thymus under conditions of substrate-competitive inhibition. Substances added to supramolecular complexes: olive oil+lipase (*1*), lipase (*2*), commercial DNA+lipase (*3*), commercial DNA+endonuclease (*4*), olive oil+endonuclease (*5*), and endonuclease (*6*). Experimental conditions: 5 ml total volume (30 μg/ml complexes in 0.14 M NaCl, pH 7, 2 μg/ml lipase, 0.3 U/ml endonuclease, 100 μg/ml commercial DNA, 5 μg/ml olive oil, 0.02% sodium azide), 37°C.

DNA degradation by lipase (Table 3). Hence, lipase is the only enzyme responsible for DNA degradation.

Of particular interest was a comparative elastoviscosimetric EB titration of nucleoid from cells (intact and fixed in 70% ethanol) preincubated with different enzymes: pronase P, RNase-A, pancreatic lipase, and phospholipase C. Figure 2 shows that titration of nucleoid from intact cells (1-h lysis) yields a typical two-phase response curve, which attests to the presence of supercoiled DNA. Two-phase curves were also observed after a 24-h lysis;

TABLE 1. Elastoviscosimetric Titration of DNA-PNM with Ethidium Bromide (EB) before and after Incubation with Pancreatic Lipase (*M±m*)

Source	EB, 6 μg/ml	Standardized viscosity, dl/g	
		control	experiment
Sarcoma 37 cells	—	2100±100	40±3
	+	4050±205	82±6
Zeidel ascitic hepatoma	—	1500±70	35±3
	+	3200±150	62±5

Note. Sample (5 ml) contains: 40 μg/ml DNA in 0.14 M NaCl, pH 7, 10 μg/ml lipase (1 h, 37°C), than 1 mg/ml pronase immediately followed by lysing mixture (1 h lysis, 24°C).

TABLE 2. Elastoviscosity of Nucleoid DNA Isolated from Cells Fixed in 70% Ethanol before and after Their Incubation with Pancreatic Lipase (*M±m*)

Cells	Standardized viscosity, dl/g	
	control	experiment
Sarcoma 37 cells	2310±150	200±10
L1210	1600±85	170±7
P388	1800±94	160±9
Loach sperm	2500±120	220±12
Loach erythrocytes	2210±105	212±10

Note. Sample (5 ml) contains: 5 μg/ml DNA in 0.14 M NaCl, pH 7, 10 μg/ml lipase (1 h, 37°C), than 1 mg/ml pronase immediately followed by lysing mixture (24 h lysis, 24°C).

however, elastoviscosity of DNA in 1-h lysates increased, indicating decompactization of the nucleoid. We have previously demonstrated [2] that treatment of leukemia L1210 cells with pronase P and RNase-A induces decompactization (increases elastoviscosity) of the nucleoid but had no effect of DNA supercoiling. This drove us to a conclusion that conformation of supercoiled DNA domains is resistant to pronase treatment. Unlike pronase, low concentrations of ethanol (25-100 mM) reduced elastoviscosity, but had no effect on supercoiled DNA structures [2]. In this context, of special importance is a phenomenon observed in our previous experiment: 20-min incubation of mice thymocytes in the presence of 9.6% ethanol partially reduced relaxation of nucleoid in the main peak of EB titration curve [12].

Other changes were observed in nucleoid from sarcoma 37 cells fixed with 70% ethanol. Figure 2 shows that fixation results in decompactization and complete relaxation of supercoiled DNA, as evidenced by the absence of two-phase responses on EB titration curves. This is apparently due to extraction of specific lipids/lipoproteins involved into stabilization of the conformation of supercoiled DNA during ethanol extraction, which confirms our previous data [9,10]. It should be noted that pronase P, RNase-A, and even phospholipase C have no marked effect on nucleoid of fixed cells. However, these cells presumably retain tightly bound lipids, since lipase considerably reduces elastoviscosity of nucleoid DNA, which is probably due to its fragmentation. The mechanisms of this effect remains unclear.

TABLE 3. Elastoviscosity of Nucleoid DNA Isolated from Sarcoma 37 Cells Fixed in 70% Ethanol Incubated with Lipase and Endonuclease in the Presence of 0.005 M EDTA

Enzymes, μg/ml	Standardized viscosity, % of control	
	without EDTA	with EDTA
Lipase		
10	19	14
15	14	16
20	11	12
Bacterial endonuclease		
10	35	102
20	14	104

Note. Incubation conditions as in Table 2. EDTA was added to enzymes before incubation with cells.

Thus, experiments *in vitro* with isolated DNA-SC and DNA-PNM and with whole cells demonstrated the presence of a lipase-sensitive component, structural neutral lipids, which along with phospholipids, proteins, and RNA are involved into compactization of eukaryotic DNA.

In the present study we attempted to demonstrate that diglycerides occupy a special place among other neutral lipids. Let us consider arguments for this assumption.

1. Neutral lipids were identified in chromosomes [7,8], chromatin [6,13], nuclear matrix [4,10] and DNA [9,10].

2. Unlike phospholipase C, pancreatic lipase eliminates triglycerides from chromosomes and modifies their structure [7,8].

3. DNA-bound lipids do not result from contamination of the preparation with membrane components during DNA isolation from nuclei [13] and whole cells [10].

4. DNA-bound free fatty acids and diglycerides are not the products of triglyceride hydrolysis [18].

5. Diglycerides tightly bound to DNA were found not only in eukaryotic cells (% of DNA [10]: rat thymus - 0.09, rat liver — 0.21, loach sperm — 0.07, sarcoma 37 cells — 0.15, Zeidel ascitic hepatoma — 17), but also in prokaryotes: *E. coli* (0.04) and phage T2 (0.04).

6. Despite a rigorous procedure of DNA isolation from chromatin (trichloroacetic acid, 80°C), diglycerides (about 20% of neutral lipids) remain bound (probably, via covalent bonds) with DNA [13].

7. It is shown that about 20% ^{3}H-glycerin are incorporated *in vivo* into DNA-bound diglycerides [13].

8. Rat liver contains natural nucleotide-diglyceride complexes [16].

9. Neutral lipids tightly bound to DNA and DNA-PNM are enriched with diglycerides (26 and 30% of total DNA-bound lipids, respectively [10]), which can be attributed to their involvement into DNA loop binding to the nuclear matrix. This is confirmed by the fact that unlike other DNA-bound

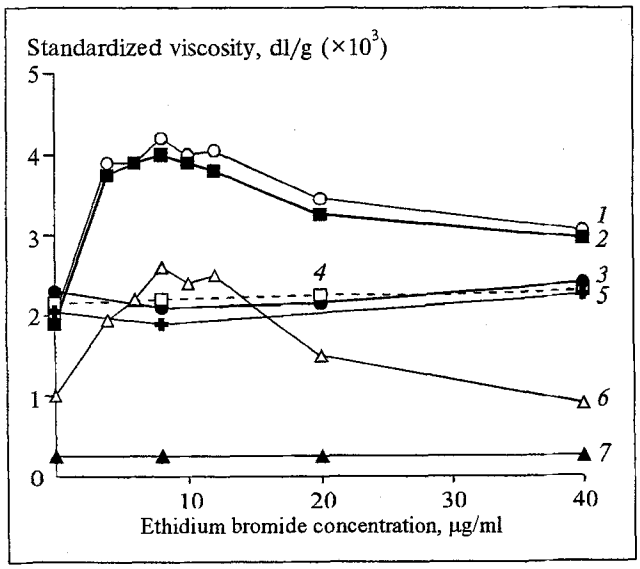

Fig. 2. Curves of elastoviscosimetric ethidium bromide titration of nucleoids from intact and 70% ethanol-fixed cells sarcoma 37 cells preincubated with different enzymes. *1*) intact cells, 24-h lysis; *2*) intact cells+pronase, 24-h lysis; *3*) fixed cells+RNase-A, 24-h lysis; *4*) fixed cells+phospholipase C, 24-h lysis; *5*) fixed cells+pronase, 24-h lysis; *6*) intact cells; 1-h lysis; *7*) fixed cells+lipase, 24-h lysis. Total volume 5 ml (5 μg DNA/ml): 100 μl cell suspension+4.9 ml lysing mixture. Incubation with enzymes: 100 ml cell suspension+100 μl enzyme (1 h, 37°C)+4.8 ml lysing mixture (1 mg/ml pronase was added to fixed cells prior to lysing mixture). Concentration of enzymes: lipase 10 μg/ml, pronase 1 mg/ml, RNase-A 0.2 mg/ml, phospholipase C 10 μg/ml).

lipids, the content of DNA-bound diglycerides remained unchanged during DNA conformational transitions from supercoiled to relaxed form [10,11]. At present, little is known on the nature of lipid-DNA bonds. In particular, cardiolipin can bind to DNA via its polar group, which sterically resembles phosphodiester framework of DNA molecule [15]; sphingomyelin can bind to DNA via an ionic bond between trimethylammonium and phosphate groups [17]. Neutral lipids, in particular, diglycerides probably bind to phosphate group of DNA molecule through its hydroxyl [16]. It cannot be excluded that diglycerides tightly bound to DNA are the recognition sites for protein kinase C localized near the basis of DNA loops and activated by diglycerides. Comprehensive evaluation of the role of diglycerides in structural organization of DNA requires additional experiments, in particular, analysis of lipid composition of DNA-SC treated with lipase, which will be the subject of our next report.

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