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Specific natural DNA-bound lipids in post-genome era. The lipid conception of chromatin organization

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Abstract

Two pools of DNA-bound lipids were isolated from DNA supramolecular complex (SC-DNA): loosely bound (extracted with 35% ethanol) and tightly bound lipids (extracted after additional treatment DNase I). The compositions of the two lipid pools from different sources (rat thymus, liver, loach sperm, pigeon erythrocytes, Zajdel ascites hepatoma, Ehrlich ascites carcinoma, sarcoma 37, *Escherichia coli* B and T2 phage) were studied. The possible functions of DNA-bound lipids, especially of cardiolipin and cholesterol, at the attachment of DNA loops to the nuclear matrix, in DNA replicon organization, replication and transcription are discussed.

Keywords: DNA; Chromatin; Cardiolipin; Cholesterol; Functional genomics

1. Introduction

Neutral lipids and phospholipids are not only components of the nuclear membrane, but they also represent important components of a number of intranuclear genetic structures, including chromosomes, chromatin and nuclear matrix [1,2]. The natural DNA-bound lipid complexes were isolated for the first time from rat thymus and liver in the authors' laboratory in 1974 [3]. These types of complexes were then isolated from other mammalian cells, tumor cells, bacteria and phages [4–6]. Firstly, the area of research of natural DNA–lipid (membrane) complexes is at present very acute because of importance of general principles of their structure and function in the development of new nonviral gene delivery systems for gene therapy purposes. Secondly, the natural DNA-bound lipids are considered now, in the post-genome era, to be involved in regulation of gene expression along with proteins and methylation of nucleic acid bases [6]. This paper is aimed to analyze the data on structural and functional role of natural DNA-bound lipids in various levels of DNA structural organization in chromatin. The lipid conception of chromatin organization is proposed here for the first time.

2. Experimental

2.1. Objects

Rat thymus and liver, regenerating liver, pigeon erythrocytes, loach sperm, Ehrlich ascites carcinoma, Zajdel ascites hepatoma, sarcoma 37, *Escherichia coli* B and phage T2.

2.2. Soft phenolic method

Soft phenolic method was used for isolation of highly polymeric DNA supramolecular complexes (SC-DNA) [7,8]. It includes very soft lysis of cells or nuclei with phenol-saturated (66%) aqueous solution at pH 8.5 (triple-repeated phenolization with 15 min of shaking followed by centrifugation at 6000 rpm/min). It results in extraction of 97% of total lipids and proteins and dialysis of final viscous DNA solution (against physiological solution) instead of its precipitation with ethanol. Final DNA solution was treated

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with RNases A or T1 to remove RNA, followed by phenolization procedure and dialysis. This method results in lipid-bound DNA preparations with very high viscosity and molecular mass (3×10^8–3×10^9 Da), and concentrations not exceeding 100–120 μg/ml. Depending on the source, the SC-DNA preparations, isolated with the method above, contain 0.1–3% of acidic nonhistone proteins (NHP), 0.1–3% of specific lipids and 0–15% of RNA [5].

2.3. Lipids

Lipids were extracted from the SC-DNA by a variety of techniques including a classical method [9] using the chloroform/methanol (2:1) mixture and separated by thin layer chromatography (silicagel H) in appropriate solutions: hexane/ethyl ether/acetic acid=73:25:2 (for neutral lipids) and chloroform/methanol/water=65:25:4 (for phospholipid separation) [5].

2.4. Experimental methods

The method of X-ray diffraction, circular dichroism, microcalorimetry, electron microscopy, viscoelastometry and sedimentation were used in this study as described elsewhere [5].

3. Results and discussion

Our study established for the first time that the extraction with chloroform/methanol (2:1) mixture removes only a part of the lipids from SC-DNA. We demonstrated that lipids in SC-DNA are presented as two pools: the pool of lipids loosely bound to the DNA (~60%), and the pool of lipids tightly bound to the DNA (~40%). The loosely bound lipids are extracted from the SC-DNA under mild treatment: with 35% aqueous solution (24 h, 37 °C without stirring). In contrast, the tightly bound lipids can be extracted with the chloroform/methanol (2:1) mixture only after incubation of residual DNA with DNase I (1 h, 37 °C).

3.1. Composition of DNA-bound lipids in eukaryotes and prokaryotes

We found that the DNA-bound lipids of eukaryotic and prokaryotic cells contain neutral lipids and phospholipids [3–6]. Phospholipids from eukaryotes consist of at least five individual fractions: cardiolipin, CL (40–50% of total phospholipids), phosphatidylethanolamine, PE (25–30%), phosphatidylcholine, PC (15–20%), phosphatidylserine, PS (5–7%), phosphatidylinositol, PI (3%) and only traces of sphingomyelin, SM, or phosphatidic acid, PA. In opposite, DNA-bound phospholipids from *E. coli* B and phage T2 are only presented by CL (60–70%) and PE (30–40%). The typical composition of DNA-bound phospholipids is presented for rat thymus in Fig. 1a.

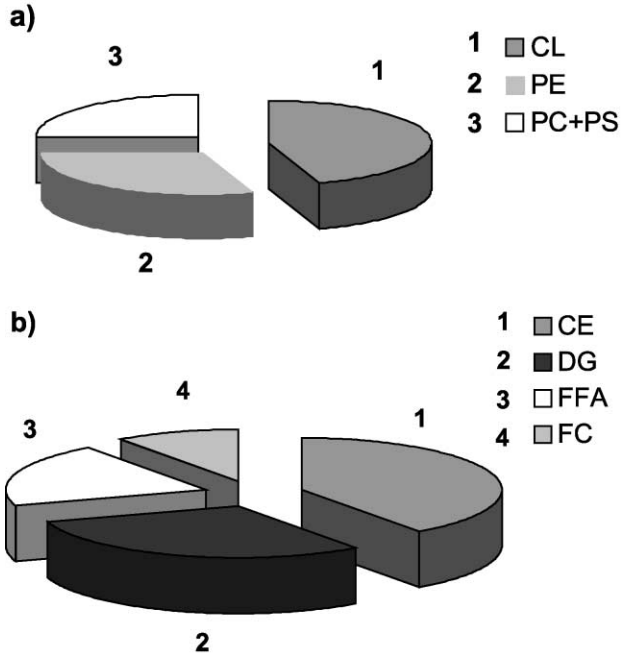

Fig. 1. Composition of DNA-bound lipids of rat thymus, in percentage of total phospholipids (CL 45%, PE 30% and PC+PS 25%) (a) or neutral (CE 40%, DG 30%, FFA 20% and FC 10%) (b) lipids.

Neutral lipids from normal eukaryotic cells, *E. coli* B and phage T2 contain four individual fractions: fatty acid esters of cholesterol, CE (38–40%), free fatty acids, FFA (20–25%), diglycerides, DG (25–30%) and free cholesterol, FC (8–10%) (Fig. 1b). Furthermore, we showed that the DNA from three types of transformed (cancer) cells contain additional fractions: triglycerides (3%) and monoglycerides (3%). Thus, the DNA-bound lipids have an unusual specific composition which differs essentially from those of nuclear membrane, chromatin and nuclear matrix lipids, where the major lipids are presented by cholesterol, PC and SM [2].

3.2. Characteristic features of DNA-bound lipids

(1) The DNA-bound lipids consist of two pools differing in tightness and the nature of their binding to the DNA molecule: loosely bound lipids and tightly bound lipids. These two pools of natural DNA-bound lipids have different quantitative lipid composition and different fatty acid composition [3–5,10]. Two different metabolic pools of lipids are also revealed in rat liver nuclear membrane and chromatin (FC, CL and SM), which in turn contain different (pH-optimum and K_m values) lypolitic enzymes (sphingomyelinase, SM synthase and PC-dependent phospholipase C) [11,12]. Besides, it is shown [13], that there are in fact two distinct subnuclear pools of diglycerides in rat liver: the first one which is highly saturated and monounsaturated (90% of total nuclear diglycerides) and the second one which is highly polyunsaturated. The authors suggest that the pools are independently regulated, possibly, by two different nuclear phospholipases C.

(2) Neutral lipids dominate over phospholipids (Fig. 2), contain considerable amount of diglycerides and free fatty acids and fatty acid esters of cholesterol dominate over free cholesterol.

(3) Cardiolipin is the major phospholipid, whereas PC is the minor one. Free cholesterol is a minor neutral lipid.

(4) There are equal contents (percentage of total lipids) of cholesterol and cardiolipin.

(5) Almost all of chromatin cardiolipin (100%) and cholesterol (60%) are localized in the DNA.

Based on the data presented, we conclude that specific natural DNA-bound lipids are of chromosomal origin and represent an integral part of DNA.

3.3. The arguments (our own results and/or literature data) in favour of structural lipids in DNA. Possible functions of DNA-bound lipids

Small-angle X-ray scattering analysis of SC-DNA of rat thymus and loach sperm revealed two equatorial reflexes (21.4 and 60 Å): the second reflex disappeared after DNA delipidization with 35% aqueous ethanol solution (the reflex at 60 Å was not observed with commercial DNA of calf thymus, Worthington, USA) [5,14]. Analogous reflex at 60 Å due to lipids was observed with calf thymus nucleohistone by Pardon and Wilkins [15]. Our studies of the dependence of Bragg's distances of these reflexes on DNA concentrations suggested that the SC-DNA is essentially the fibrils [14], each with four DNA molecules packed side-by-side and/or end-to-end by lipids. In support of this, our electron microscopy studies revealed DNA fibrils 50–70 Å in diameter consisting of four DNA molecules [16] and also loop- and rosette-like DNA structures with compactization centers, which were converted to linear duplexes 20 Å in diameter after incubation with pancreatic lipase [5,17].

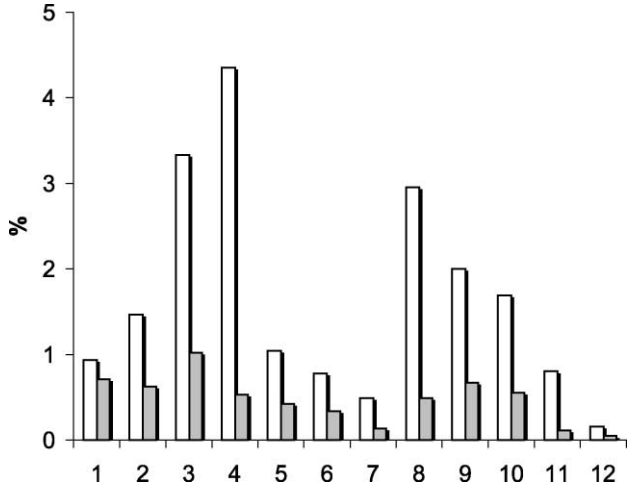

Fig. 2. Contents of DNA-bound neutral lipids (gray) and phospholipids (black) of SC-DNA of various eukaryotic and prokaryotic cells (percentage to DNA, w/w). (1) Rat thymus; (2) rat liver; (3) regenerating liver, S-phase; (4) regenerating liver, G_2-phase; (5) loach sperm, supercoiled DNA; (6) loach sperm, relaxed DNA; (7) pigeon red blood cells; (8) Zajdel ascites hepatoma; (9) sarcoma 37; (10) Ehrlich ascites carcinoma; (11) *E. coli*; (12) T2 phage.

Using the circular dichroism method (CD), it was shown that SC-DNA from actively transcribed cells (thymus, spleen, kidney, rat brain and Ehrlich ascites carcinoma) have, as a rule, A-form, while SC-DNA from repressed genome (pigeon erythrocytes and loach sperm) occurs in B-form [5]. Through special experiments, it was discovered that DNA conformation change is due to lipids, in particular, cardiolipin [18].

The method of adiabatic scanning microcalorimetry revealed the jump in heat capacity on heat absorption curves at 200–300 kJ/kg degree in 20–25 °C interval for SC-DNA from rat thymus and loach sperm (with commercial DNA, such a jump did not occur) [5]. It is important that this surge disappeared only after treatment with lipase or phospholipase.

Addition of unlabeled and (2-^{14}C-acetate) labeled total rat liver lipids to cell homogenate prior to SC-DNA isolation did not change the lipid contents and did not reveal any radioactivity in the complex [5]. The amount of tightly bound lipids in DNA from Ehrlich ascites carcinoma cells does not depend on either the method of DNA isolation or on the isolation from cells, nuclei or chromatin [5,19]. Thus, by various biochemical, physico-chemical and physical methods, it was proven that lipids (lipoproteins) are involved at various levels of supramolecular organization of DNA.

3.4. Lipid concept of chromatin organization

Thus, the data discussed above are summarized here as a lipid contribution to the protein concept of chromatin organization, which could be important along with considering the role of specific nonhistone proteins.

(1) Chromatin lipids: cardiolipin, diglycerides, cholesterol and its ethers—play the key role in the supramolecular organization of chromatin [2,4–6].

(2) There exist two pools of DNA-bound lipids: loosely bound and tightly bound ones. Qualitative and quantitative compositions of the two pools depends on the object's nature, genome activity, on the phase of cell cycle and the presence of malignancy. Compositions of the two lipid pools distinct, in principal, with contents of cholesterol, cardiolipin and phosphatidylcholine. The loosely bound lipids may act as linkers between DNA replicon transcription, whereas the tightly bound lipids may be the specific sites of attachment of DNA loops to the nuclear matrix [4,5].

(3) Anionic phospholipids (cardiolipin and phosphatidylserine), in contrast to amphiphilic and cationic phospholipids (phosphatidylcholine and phosphatidylethanolamine), promote chromatin decondensation, substitute H1 histone from linker DNA, induce transition of chromatin from solenoid to nucleosome conformation and activate RNA polymerase [20].

(4) The ratio of neutral lipids to phospholipids into DNA, increases dramatically (by 2–3-fold) during malignant transformation (Fig. 2), however, the contents of cardiolipin are not changed, but the new lipid fractions (mono- and triglycerides) appear [5].

(5) The lipids of chromatin/DNA play a structural role (diglycerides and cholesterol), a functional role (cardiolipin and sphingomyelin), and a structural–functional role (cardiolipin and sphingomyelin) [2].

(6) Cardiolipin has a common antigenic ''interphosphate'' moiety with DNA [21]. All amounts of chromatin cardiolipin and cholesterol (60%) are bound to the DNA [5]. Cardiolipin is the phospholipid of proliferation and dominates in ''active'' genomes [6]. Cardiolipin regulates activity of DNA topoisomerase II [22], which is localized just at the DNA loop attachment site on the nuclear matrix, e.g., at the replication initiation site. Cardiolipin regulates the DNA replication, replicative proteins—DNA A protein *E. coli* and SV-40 T-antigen (animals) being activated [2,23]. Cardiolipin specifically abolishes the inhibitory effect of histones on chromatin transcription and modulates the binding of histones to DNA [24]. Cardiolipin provides the DNA B-form-to-A-form transition in the complex with RNA polymerase 1, which is necessary for the transcription [2,18].

(7) The lipids of SC-DNA and chromatin (cardiolipin and cholesterol) are the targets for ionizing radiation and antitumor drugs, they are responsible for DNA degradation through free radical mechanism [5] in the chromatin/DNA.

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