Cholesterol and its fatty acid esters in native DNA preparations: lipid analysis, computer simulation of their interaction with DNA and cholesterol binding to immobilized oligodeoxyribonucleotides

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Supramolecular DNA complexes were isolated from rat normal cells and murine tumors. The content of DNA-bound lipids (cholesterol and its esters) was determined. The content of cholesterol esters is higher than that of free cholesterol; the lipid content in tumor cells is higher than in normal cells. Using the molecular mechanics approach, it is demonstrated for the first time that cholesterol and its esters with stearic, oleic, linoleic, and linolenic acids bind to the DNA minor groove more strongly than with the major groove. The calculated DNA binding energies of cholesterol and its esters depend on both the number of double bonds in the fatty acid residue and on the DNA nucleotide composition. The formation of stable complexes between cholesterol molecules and d(AT)-rich oligonucleotides was demonstrated using biological microchip containing immobilized octadeoxyribonucleotides.

Key words: supramolecular DNA complexes from eukaryotes, cholesterol, cholesterol esters, biochips, computer simulation.

Lipidomics is a field of knowledge supplementing genomics and proteomics and concerned with systematic analysis of the structure, properties, and functions of cell lipids.[1—4] Eukaryotic cells, first of all, human cells contain more than a thousand classes of lipids. This impressive diversity is attained by incorporating various polar heads and hydrophobic acyl (alkyl) residues in various combinations into the lipid structure. The structural lipidomics deals with the composition, the structure, and the properties of structural, functional, and, to some extent, reserve lipids connected to the biomacromolecules of cell organelles and membranes.[2,3] The functional lipidomics studies the nature and functions of the membrane and cell lipids (functional, signaling, and group-active, *i.e.*, those characterizing the blood group) in the biosynthesis of biologically active molecules, signal transfer, gene expression, and so on.[3] A goal of this field of science is to elucidate the functional causes for the diversity of lipids. Other definitions consider lipidomics as a part of metabolomics and relate it to the investigation of water-insoluble metabolites. The metabolomics (signalomics) is formulated as a field of science dealing with the structure and the level of low-molecular-weight metabolites, depending on the function of the corresponding proteins and encoding genes.[4]

Lipids including cholesterol, fatty acids, and their esters play an important structural and functional roles in DNA, chromosome, chromatin, and nuclear matrix organization and in signal transmission.[5—13] The natural supramolecular complexes of lipids with DNA were first isolated from rat thymus and liver,[14] and then from other eukaryotic cells by the phenol method.[12,15] These DNA complexes ($3 \cdot 10^8$—10^9 Da) contain acidic nonhistone proteins (1—4%) and lipids (1—4%) including cholesterol and its esters,[8,9,12,14] the content of cholesterol esters being usually 2—3 times as high as that of free cholesterol. Notably, 60% of the total fraction including cholesterol and its esters located in chromatin is bound to isolated DNA preparations.[9] Two fractions of DNA-bound lipids are distinguished, namely, weakly and strongly bound lipids, their ratio being dependent on the stage of the cell cycle and gene expression and malignant trans-

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formation levels. Currently, it has been shown that cholesterol is firmly bound to chromatin,[16] serves as a signaling molecule,[6] and represses the transcription of the SCD human gene[17] by participating in the gene expression regulation. Cholesterol or its metabolites might also be involved in the formation of DNA—protein—lipid ternary complexes. Of particular interest is the fact that the composition of the fractions of DNA-bound cholesterol and its esters may vary in morbid conditions.[8,11—13,18] Note that the contents of cholesterol and its esters with fatty acids (stearic, oleic, linoleic, and linolenic acids), the nature of their binding in the DNA complex, and their function in chromatin have not been adequately studied.

The currently existing lipidomics projects,[4] in conformity with the lipid isolation protocol used, do not stipulate the analysis of the DNA-bound lipids. Therefore, we consider the study of these lipids highly topical. Our previous studies were devoted to analysis of the fatty acid fraction of the pool of neutral lipids bound to the eukaryotic DNA.[18,19] This communication presents the data on the content of cholesterol and its esters with fatty acids in DNA-bound lipids of some eukaryotic cells including comparison of the cells in the normal condition and upon malignant transformation. Studies of the nature of DNA binding of cholesterol and cholesterol esters with fatty acids have been pursued along two directions. DNA hybridization (on biochips) allows one to estimate the specificity of cholesterol interaction with octadeoxyribonucleotide. Computer simulation of the interaction of DNA oligomer duplexes with cholesterol and cholesterol esters with fatty acids was carried out by the molecular mechanics approach.

Experimental

The study was carried out with ascite tumor cells (Zeidel ascite hepatoma, sarcoma 37, and Erlich ascite carcinoma) on the 7th day after the intraperitoneal subinoculation in white nonlinear male mice weighing 20—25 g from the farm of the Russian Academy of Medical Sciences. After animal decapitation, ascite cells were withdrawn, washed with 0.14 *M* NaCl, and 3 mL of the crude cell precipitate was used for analysis. The cells of healthy rats of the Wistar line weighing 120—130 g on a standard diet were also used. The rats were killed on 9—10 o´clock in the morning, the thymus and the liver were homogenized in a 0.14 *M* NaCl—0.05 *M* sodium citrate solution, pH 7, at 2—4 °C in a glass homogenizer with a Teflon pestle (ten tractions). The DNA supramolecular complex (scDNA) was isolated from rat tissues and tumor cells using the mild phenol method,[12,15] which includes treatment with 66% aqueous phenol, pH 8.5 (1 : 1) repeated three times, which removes ~97% of proteins and lipids. Phenol was removed from the solution of scDNA by dialysis against 0.14 *M* NaCl at 4 °C. The content of DNA was determined using the Dische diphenylamine reagent.

Analysis of the DNA-bound lipids. The lipids from scDNA were isolated and analyzed by a reported procedure.[9] The fraction of weakly bound lipids (fraction I) was obtained by treatment of scDNA with a 35% aqueous ethanol as follows: 96% ethanol (51 mL) was added in portions to 100 mL of a solution of scDNA (90 μg mL^{-1}); after 24 h at 37 °C, two volumes of cold 96% ethanol were added, the precipitate was washed three times with 70% ethanol, and the ethanolic extracts were combined (fraction I). The scDNA precipitate formed after treatment with ethanol was dissolved in 5 mL of 0.14 *M* NaCl, DNAse I (300 μg) (Kochlight, DNA : DNAse = 10 : 1) in 0.01 *M* $MgCl_2$ (5 mL) was added, and the mixture was incubated for 2 h at 37 °C. The strongly bound lipids (fraction II) were extracted with a chloroform—methanol mixture (2 : 1) using the Folch procedure (1 : 1 v/v), and the extracts were concentrated *in vacuo* at 40 °C.

The lipid fractions were analyzed by TLC on silica gel H (Merck) in the following solvent systems: for neutral lipids, hexane—diethyl ether—acetic acid (73 : 25 : 2); for phospholipids, chloroform—methanol—water (65 : 25 : 4). The lipids were identified using analytically pure standards (Analab, Inc., UK), the substances were visualized by iodine vapor. The silica gel zones with particular R_f were transferred into quartz tubes and heated in concentrated sulfuric acid for 15 min at 200 °C; the optical density was determined at 375 nm. The results were processed using the Student *t*-criterion and are presented as average values of three to five runs with root-mean-square deviations.

Study of the specificity of cholesterol interaction with DNA using biochips. Biochips with immobilized single-stranded octadeoxyribonucleotides were used.[20] A biochip (DNA microarrays) is a glass plate with cells of polyacrylamide gel each containing one oligonucleotide[21] (Fig. 1). Oligonucleotides bearing the amine linker $C_7H_{14}NH_2$ at the 3´-end were attached to the gel containing aldehyde groups by means of reductive amination.[20—22] The sequences of all oligonucleotides represented a hexameric core, differing in composition, flanked on both sides by a statistic mixture of four nucleotides, for example, 5´-N-d(GCGCGC)N-3´-$C_7H_{14}NH_2$ or 5´-N-d(ATATAT)N-3´-$C_7H_{14}NH_2$, where N is any of the four deoxynucleotides (A, T, G, or C). Thus, the physical length of each oligonucleotide was eight, but hexamers were the significant fragments for the recognition of complementary sequences. In the case of statistical flanking, the influence of the terminal nucleotides is averaged and, therefore, it plays no role in recognition. The sequences of the given biochip and the flanking nucleotides were chosen considering the least cross-hybridization. A mixture

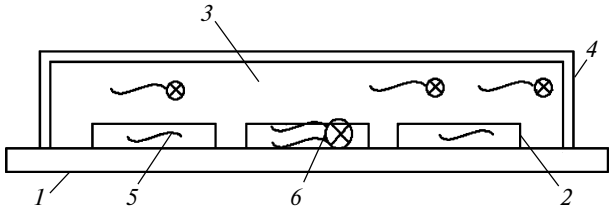

Fig. 1. Scheme of a biochip for hybridization of oligodeoxyribonucleotides: (*1*) glass plate, the biochip base, (*2*) gel cell containing immobilized oligodeoxyribonucleotides, (*3*) inner space of the chamber with a mixture of oligodeoxyribonucleotides with a fluorescent label, (*4*) hybridization chamber, (*5*) immobilized oligodeoxyribonucleotide, (*6*) the cell in which hybridization accompanied by enhancement of the luminescence has taken place.

Table 1. Sequences of oligonucleotide cores immobilized on a biochip

Core sequences, the number of AT pairs in the duplexes					
1(AT)	2(AT)	3(AT)	4(AT)	5(AT)	6(AT)
CGCCGA (1.1)	CTGGGT (1.0)	AGCCTA (1.1)	CGTTTA (0.9)	CTATTT (0.9)	TTTATT (1.7)
CGGGTG (1.3)	CGACGA (1.0)	GTGTTG (1.0)	TTTTGC (0.9)	CTTTTT (0.7)	TTTTTT (1.0)
GGGCGA (1.1)	GTCAGG (1.0)	CGACTA (1.0)	TGAAGG (0.6)	ATAGTT (0.6)	TTAATA (8.9)
CGACGC (1.1)	TTGCGG (0.9)	TTGTGC (0.8)	ATGTGT (0.8)	TTTTTG (0.8)	TTTATA (1.3)
GGTCGG (1.1)	TGCAGG (0.9)	TGGATG (0.9)	TTCGTA (0.8)	TTAATG (0.7)	ATAATT (0.2)
CGTCGC (0.9)	GTGGTG (0.9)	TGCGTA (0.8)	TGATTC (0.6)	GTAATT (0.8)	ATATTA (17.0)
GGCCGA (1.0)	CGGATG (0.8)	AGAGTG (0.7)	ATTTGG (0.6)	ATGATT (0.4)	ATTATT (0.8)

Note. The relative change in the fluorescence intensity of double-stranded nucleotides in the presence of cholesterol is given in parentheses. During hybridization, the second strand bearing the fluorescent label adds to each oligonucleotide immobilized on the biochip.

of 16 octamers having the same hexameric core but different flanking nucleotides is immobilized in each biochip cell. The change in the fluorescence intensity in the presence of cholesterol corresponds to the response of a statistical mixture of 16 octanucleotides with the same hexanucleotide core. The biochip used in this work contained 42 immobilized oligonucleotides with different compositions; the nucleotide sequences in their cores are presented in Table 1 (insignificant flanking nucleotides are not shown). This biochip is a fragment of a large "generic" biochip containing $4^6 = 4096$ possible hexamers and has been repeatedly used to study the specificity of protein and ligand interaction with DNA.[20—22]

All oligodeoxyribonucleotides were synthesized using an Applied Biosystems-394 synthesizer and standard phosphoramidite procedures. Single-stranded oligonucleotides were converted into double-stranded ones by hybridization with a mixture of octanucleotides with a fluorescent label at the 3′-end (Texas Red) whose components are complementary to all 42 matrix oligomers (see Fig. 1); the mixture concentration was 50 μmol L^{-1}. Hybridization was carried out for 12 h at 4 °C and was followed by washing-out of the remaining oligomers with a buffer solution.[20]

The specificity of cholesterol interaction with double-stranded DNA was determined in two parallel experiments. Immobilized single-stranded oligonucleotides were hybridized on a reference biochip with a mixture of fluorescence-labeled octamers without a lipid. On the second biochip (under study), the same hybridization was carried out in the presence of 50 μ*M* solution of cholesterol in ethanol. After hybridization, both biochips were washed with a buffer solution and dried. The cell fluorescence on both biochips was determined using a fluorescent microscope (LOMO, Russia) equipped with a PZS-electronic camera (Princeton Instruments, USA).[20—23] In terms of this method, the fluorescence intensity is the measure of transition of single-stranded immobilized oligonucleotides into the double-stranded form.[21—23] The degree of hybridization of biological microchips (the amount of the complementary fluorescent strand in different cells) in the control run and in the presence of cholesterol was determined by processing the data using an original program.[20]

Computer simulation of the structure and stability of the complexes. All calculations for the $d(AT)_n \cdot d(TA)_n$ or $d(GC)_n \cdot d(CG)_n$ complexes with cholesterol or its esters were carried out by the MM+ molecular mechanics method using the Hyperchem 5.01 program as described previously.[16,17] Within the framework of this method, the total energy (E_{total}) of a molecular system is represented as the sum of the contributions of the energies of chemical bond stretching (E_R) and deformation of bond angles (E_Q) with respect to the standard R_0 and Q_0 values, the torsion energy (E_{tors}), the energies of electrostatic (E_e) and van der Waals (E_{vdw}) interactions between nonbonded atoms, and hydrogen bond energy (E_H). In this empirical approach, the force field parameters are chosen in such a way as to reproduce the structure and properties of a rather broad class of organic substances.

We carried out full geometry optimization by molecular mechanics for fragments of the DNA double strand, for cholesterol (**1**), and its esters (**2—5**) with C_{18}-fatty acids (stearic, oleic, linoleic, and linolenic, respectively, which are encountered most frequently) (Fig. 2), and for the DNA—ligand complexes. The DNA—ligand binding energies E_b were determined. The calculations were carried out for DNA in the B-form without taking account of the bound water molecules. The sodium ions were added for system electric neutrality. The energy of DNA binding to the ligands was determined as the difference between the total energies of the DNA—ligand complex and the isolated molecules. Taking into account the geometric features of B-DNA, the ligand molecules were arranged in the double helix grooves, and, hence, the complexation energies of the ligand with DNA from the minor and major groove sides were estimated separately. All calculations used the fully staggered conformation for the saturated parts of fatty acid residues (see Fig. 2).

Results and Discussion

Analysis of the lipid content. Table 2 presents the contents of cholesterol and its esters in two lipid fractions in scDNA. The cholesterol content in DNA is 0.8 to 2.6 molecules and that of cholesterol esters is 1.2 to 6.6 molecules per 1000 bp. The content of cholesterol and its esters in the fraction of weakly bound lipids is higher than that in the strongly bound lipid fraction, which is especially typical of tumor cells, except for the S-phase of the regenerating rat liver. The cholesterol and cholesterol

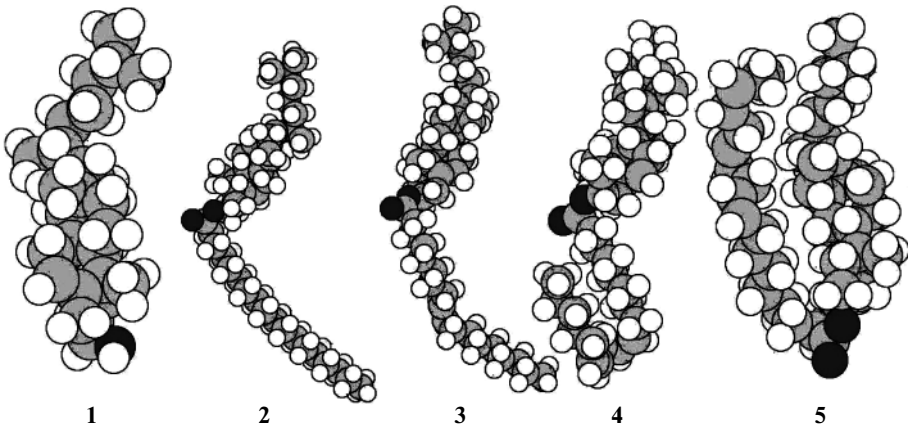

Fig. 2. MM^+-optimized configurations of cholesterol (**1**) and its esters with stearic (**2**), oleic (**3**), linoleic (**4**), and linolenic (**5**) acids.

ester contents in the fraction of weakly bound lipids is higher for tumor cells than for normal cells. It is noteworthy that the content of the fractions of weakly and strongly bound cholesterol esters in two actively expressing genomes (the hepatocyte S-phase and the Zeidel hepatoma) is markedly higher than the content of these fractions of cholesterol itself. Minor amounts of strongly bound lipids, including cholesterol and its esters, have been detected by physical, physicochemical, and biochemical experiments (*in vivo* incorporation of ^{14}C-acetate, ^{14}C-cholesterol, ^{3}H-glycerol) in DNA preparations isolated by other methods and from other objects.[8,12,16]

Complexation of cholesterol with immobilized oligodeoxyribonucleotides. Previously, all determinations of the contents of cholesterol and its esters with fatty acids in the fractions of natural DNA-bound lipids have been carried out using natural complexes. In order to supplement these data, cholesterol complexation with model objects, oligomeric fluorescence-labeled DNA-duplexes immobilized on biochips, has been studied.[20—23]

The results of experiments suitable for estimating the sequence specificity of the DNA—cholesterol interaction are given in Table 1. A noticeable influence of cholesterol on the fluorescence intensity of the duplexes was detected only for several oligodeoxyribonucleotides with high contents of the AT-pairs: TTTATT (+70%), TTAATA (+790%) and, especially, ATATTA (+1600%). Thus, the complexation of these duplexes with cholesterol stabilizes the double-stranded oligonucleotide and sharply enhances the fluorescence. In this case, cholesterol acts as a "clip". For oligonucleotides with a high content of GC-pairs, the cholesterol-induced enhancement of the fluorescence intensity (hybridization) is much less pronounced (10—20%) and falls within the experimental error. In some cases, the fluorescence intensity becomes weaker due to cholesterol, for example, by 20% for the TTGTGC oligomers, by 40%

Table 2. Content of cholesterol and its esters with fatty acids in scDNA of eukaryotic cells (averaged number of DNA-bound lipid molecules per 1000 bp (±5%))

Subject	Cholesterol		Cholesterol esters with fatty acids	
	I	II	I	II
Rat thymus	0.9	0.8	1.8	1.2
Rat liver: G_0-phase	1.4	0.7	2.4	2.0
Rat liver: S-phase	1.3	2.6	6.6	5.6
Zeidel ascite hepatoma	2.6	0.7	4.2	4.2
Sarcoma 37	2.6	1.9	6.3	1.3
Erlich ascite carcinoma	2.6	0.5	2.4	2.1

Note. (I) weakly bound lipid fraction, (II) strongly bound lipid fraction.
The following average molecular masses were taken for calculating the number of bound cholesterol molecules (based on the mass content of cholesterol in DNA determined experimentally): DNA, 10^8; cholesterol, 386.6; and cholesterol esters, 600 Da (assuming that cholesterol esters in the obtained natural preparations may represent mixtures of C_{10}—C_{18} and higher homologs of fatty acids).

for the TGAAGG, TGATTC, and ATTTGG oligomers, and by 60% for the ATGATT oligomers.

Thus, the interaction of DNA with cholesterol is confirmed for the DNA fragments known to contain no other biomacromolecules; it is observed only for AT-rich DNA and appreciably depends on the nucleotide sequence.

Stability of the DNA complexes with cholesterol and its esters. Computer experiments. In previous studies, we discussed the results of investigations of the DNA interaction with fatty acids carried out by biochemical, biophysical, and computer experiments.[16,17] For calculations of complexes of fatty acids with DNA fragments, we chose the $d(AT)_n \cdot d(TA)_n$ and $d(GC)_n \cdot d(CG)_n$ oligodeoxyribonucleotides (n = 5, 7). It was shown by molecular mechanics that fatty acids can be bound more strongly to the DNA minor groove than to the major groove. The energy and the type of interaction of fatty acids with DNA depends on both the number and configurations of the double bonds and the nucleotide composition of DNA.

Previously, when titrating homo- and heteropolydeoxyribonucleotides with oleic acid by the CD method, we found a dependence of the degree of complexation on the nucleotide sequence.[10] In particular, polyd(A)·polyd(T) and polyd(AT) tend to interact according to the recognition pattern: the CD spectrum changes jumpwise upon binding of one oleic acid molecule per DNA step and subsequently it does not change on an increase in the amount of the ligand. Conversely, an increase in the fraction of oleic acid in the titration of $polyd(GC)_n$ (from 1 : 10 to 1 : 1 per bp) induces a gradual decrease in the positive band at 260 nm and a synchronous increase in the amplitude of the band 280 nm.

The calculations for the complexes of ligands **1—5** with DNA fragments were carried out using double-stranded decadeoxyribonucleotides, $d(AT)_5 \cdot d(TA)_5$ and $d(GC)_5 \cdot d(CG)_5$ (DNA). The total energies E_{total} of the DNA $d(AT)_5$, $d(GC)_5$ fragments and fatty acid molecules have been reported in our previous publications.[16,17] The total energies of ligands **1—5** are 59.1, 73.6, 73.5, 72.7, and 63.8 kcal mol^{-1}, respectively. The positions of ligands in DNA grooves is illustrated in Fig. 3, which shows the optimized structures of $d(GC)_5 \cdot d(CG)_5$ complexes with cholesterol (**1**) and its ester with oleic acid (**3**) in the minor and major grooves. Comparison of Figs 2 and 3 is indicative of a change in the lipid conformation upon the complexation with DNA.

Consideration of the calculated binding energies of cholesterol **1** and its esters **2—5** shows that cholesterol forms complexes with DNA with approximately the same stabilities (16.2—38.1 kcal mol^{-1}) as fatty acids (Table 3). The interaction of cholesterol with the $d(AT)_5 \cdot d(TA)_5$ duplex is stronger than with the $d(GC)_5 \cdot d(CG)_5$ duplex, which is consistent with the data obtained on biochips according to which high content of AT-pairs is favorable for the strong DNA—cholesterol interaction. Transition

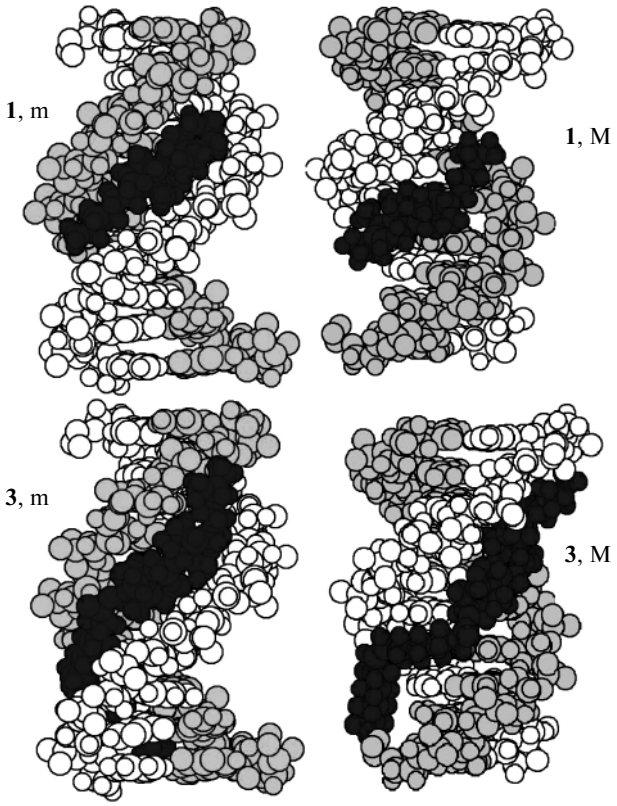

Fig. 3. Equilibrium geometry of the $d(GC)_5 \cdot d(CG)_5$ complexes with cholesterol (**1**) and its esters with oleic acid (**3**) in the minor (m) or major (M) groove.

to cholesterol esters is accompanied by stabilization of complexes with ligand arrangement in the DNA minor groove. For example, the complexation energy of $d(AT)_5 \cdot d(TA)_5$ with cholesterol **1** in the DNA minor groove is 38 kcal mol^{-1}, while the energies of DNA complexes with cholesterol esters **2—5** in the same groove are higher, namely, 58, 46, 45, and 51 kcal mol^{-1}, respectively (see Table 3). In the case of the $d(GC)_5 \cdot d(CG)_5$ complexes with cholesterol and its esters located in the minor groove, transition from the cholesterol complex to the complexes of its esters is accompanied by an increase in the binding energy from 30 for **1** to 67, 66, 48, and 47 kcal mol^{-1}, respectively, for cholesterol esters **2—5**. It can be seen that the binding energy increases 1.5—2-fold on passing from cholesterol to its esters, which is in line with the additivity principle according to which the binding energy of cholesterol ester with DNA (E^1_b) is approximately equal to the sum of the cholesterol (E^2_b) and fatty acid (E^3_b) binding energies with DNA.

$$E^1_b \approx E^2_b + E^3_b, \quad (1)$$

i.e., in the complexes with cholesterol esters, the cholestane core and the fatty acid residue interact almost independently with the minor groove of DNA; for cholesterol esters, this interaction is summed up.

Table 3. Energies of formation of DNA complexes with cholesterol and its esters for ligand arrangement in the minor (m) or major (M) grooves

Complex composition	Arrangement in the groove	E_{total}	E_b
		kcal mol^{-1}	
$(AT)_5(TA)_5$-**1**	m	683.6	38.1
$(AT)_5(TA)_5$-**1**	M	687.9	33.9
$(GC)_5(CG)_5$-**1**	m	392.3	30.1
$(GC)_5(CG)_5$-**1**	M	406.2	16.2
$(AT)_5(TA)_5$-**2**	m	677.0	58.3
$(AT)_5(TA)_5$-**2**	M	704.1	31.2
$(GC)_5(CG)_5$-**2**	m	376.4	67.1
$(GC)_5(CG)_5$-**2**	M	406.4	37.1
$(AT)_5(TA)_5$-**3**	m	689.5	45.7
$(AT)_5(TA)_5$-**3**	M	705.7	29.5
$(GC)_5(CG)_5$-**3**	m	377.3	66.1
$(GC)_5(CG)_5$-**3**	M	403.9	39.5
$(AT)_7(TA)_7$-**3**	m	951.2	64.7
$(AT)_7(TA)_7$-**3**	M	974.0	41.9
$(GC)_7(CG)_7$-**3**	m	537.6	56.6
$(GC)_7(CG)_7$-**3**	M	551.4	42.8
$(AT)_5(TA)_5$-**4**	m	689.9	45.3
$(AT)_5(TA)_5$-**4**	M	695.1	40.1
$(GC)_5(CG)_5$-**4**	m	386.4	47.6
$(GC)_5(CG)_5$-**4**	M	406.3	29.6
$(AT)_5(TA)_5$-**5**	m	674.8	50.7
$(AT)_5(TA)_5$-**5**	M	692.0	33.5
$(GC)_5(CG)_5$-**5**	m	387.2	46.5
$(GC)_5(CG)_5$-**5**	M	387.5	46.2

Note that the results of calculations of the complexation energies of cholesterol esters with DNA do not depend, on the qualitative and semiquantitative levels, on the length of the chosen DNA fragment. However, the calculated binding energies for these complexes for double-stranded tetradecanucleotides $d(AT)_7 \cdot d(TA)_7$ and $d(GC)_7 \cdot d(CG)_7$ are higher than those for $d(AT)_5 \cdot d(TA)_5$ and $d(GC)_5 \cdot d(CG)_5$ (by 50%), although the pattern of variation of the values does not change. Therefore, all calculations carried out for decaoligonucleotides, $d(AT)_5 \cdot d(TA)_5$ and $d(GC)_5 \cdot d(CG)_5$, give supposedly the lower estimate for the binding energies of the esters with DNA.

In the major groove of $d(GC)_5 \cdot d(CG)_5$, the replacement of cholesterol by its esters induces an approximately twofold increase in the binding energies of the complexes (from 16 to 30—46 kcal mol^{-1}) depending on the structure of the fatty acid, in conformity with the additivity rule (1). This scheme is violated only for the case of binding of cholesterol or its esters in the major groove of the DNA-duplex, $d(AT)_5 \cdot d(TA)_5$, in which the binding energy is 34 kcal mol^{-1} for cholesterol and 31, 30, 40, and 33 kcal mol^{-1} for cholesterol esters. The additivity rule (1), which was obeyed in the simulation of the interaction of cholesterol esters with fatty acids to the DNA minor groove, does not hold in this case. This may be related to particular properties of the chosen nucleotide sequences, specifically, with the high degree of exposure of the methyl group of thymine in the major groove of $d(AT)_5 \cdot d(TA)_5$, which makes it more hydrophobic than the groove of the double-stranded $d(GC)_5 \cdot d(CG)_5$ oligonucleotide.

An increase in the stability of the complexes on passing from cholesterol to its esters corresponds to biochemical data obtained in *in vivo* experiments. A higher (by a factor of 1.5 to 6) content of cholesterol esters compared to cholesterol is observed for eukaryotic genomes (see Table 2). The results obtained *in silico* are consistent with the results of *in vitro* experiments. A study of the interaction of the ^{3}H-labeled plasmid DNA with cholesterol or its esters with oleic or linoleic acid has shown that the amount of cholesterol esters bound by DNA is 500 times as great as the amount of cholesterol.[7]

Thus, our experiments showed that cholesterol and its esters are present in the DNA complexes of both normal and malignant cells. The DNA-bound lipids were found to contain more cholesterol esters than free cholesterol. Tumor cells have higher contents of cholesterol and its esters bound by scDNA. Using molecular mechanics, we showed for the first time that cholesterol and its esters with stearic, oleic, linoleic, and linolenic acids are bound more strongly to the minor groove of DNA than to the major groove. The binding energy of cholesterol esters to DNA depends on both the number of double bonds in the fatty acid residue and the nucleotide composition of the DNA. The highest interaction energy between DNA and cholesterol esters is found for stearic and oleic acid derivatives located in the $d(GC)_5 \cdot d(CG)_5$ minor groove (67 kcal mol^{-1}), while the lowest interaction energy (31 kcal mol^{-1}) is typical of oleic or linoleic acids located in the major groove of the $d(AT)_5 \cdot d(TA)_5$ duplex.

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