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Effect of cholesterol on the DNA Conformation *In-Vitro.*

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ABSTRACT

The effect of cholesterol on the structure of de-lipidised DNA molecules was studied using Raman spectroscopy (RS) and nanostructured silver scaffold (NSS). The findings show the change in conformation and increase of the DNA hydrophobicity when cholesterol added.

Keywords: DNA, cholesterol, hyperchromic effect, nanostructures, plasmonic resonance.

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INTRODUCTION

The study of the molecular mechanisms of regulation of gene expression is important for an understanding of the key moments of the pathogenesis of various diseases and the development of effective methods for their treatment. In this connection it is of interest to study the influence of biomolecules performing important functions in a cell involved in the pathogenesis or disease on genetic expression.

Among these molecules is cholesterol (LDL), which operates in the cells as a biomembrane component influencing their structural and functional organization, a precursor of steroid hormones, etc. Under certain conditions, associated primarily with increased free-radical processes in the body, cholesterol, or rather its modified forms can play a pathogenic role in promoting the development of atherosclerosis [2].

Studying of the effect of cholesterol on DNA is based on the fact that in the nucleus, chromatin and genomic DNA diverse qualitative and quantitative content of lipids fund is present, which includes the free and esterified cholesterol that is divided into pools of loosely coupled and strongly bound lipids that are labile and vary depending on the functional state of the cell [5].

We have previously shown that the combined use of the NSS and the RS allows you to enhance the evaluation of conformational rearrangements of the DNA molecule in the pathology [1]. In this paper we studied the cholesterol effect on the structure and conformation of DNA in *in vitro* experiments using labeled approaches.

MATERIAL AND METHODS

DNA samples isolated from nucleated human blood cells by the method of Laura-Lee Boodram, that is based on the use of proteinase K and 10% SDS, were used as an object of the research. Purification of nucleic acid from protein was performed using 5.3 M solution of NaCl and isopropanol. DNA was precipitated with 70% ethanol and then resuspended in Tris-HCl, pH 8.5 [6]. Separation of the DNA fragments was performed in 1.5% agarose gel [7].

Raman spectroscopy (RS) of the DNA was recorded with the Renishaw's *in via Basis* spectrometer (United Kingdom). The laser with a 532 nm emission wavelength, capacity of 100 mW and a 50x lens was used for exciting. The RS was recorded with a CCD detector (1024x256 pixels with Peltier-cooling up to -70ºC) with a grating of 1800 lines / mm. In Raman spectroscopy spectral bands ranging from 600 to 2000 cm^{-1} were used. To enhance the RS the substrates with nanostructured coating based on silver were used (courtesy of G. V. Maximov and E. A. Goodilin) on which the DNA samples were applied, and the DNA had previously been dissolved in a 100 mM solution of NaCl (40 mcg/ml).

The measurement of the absorption spectrum of DNA solution was performed on a Shimadzu UV-3600 spectrophotometer.

In order to determine the interaction of cholesterol with DNA experiments were performed in two ways: firstly, cholesterol as the alcohol solution was added to the solution of DNA in phosphate buffer (the concentration of alcohol in the final solution did not exceed 0.02%); secondly, cholesterol was added to the denatured DNA at 90°C and pH 8.5.

Statistical processing was performed with STAT3 program and Microsoft Office Excel, the value of $p < 0.05$ was taken as a minimum credibility threshold.

RESULTS AND DISCUSSION

The experiment shows that the cholesterol binding to oligonucleotide sequences of DNA facilitates change of spectral properties of a solution. In control the DNA absorption peak (λ_{260}) corresponds to the value of A = 0.585. When cholesterol is added to the solution of DNA in a molar ratio of 1: 3, optical density of the solution is increased by 20% related to control. It is also noted that the addition of cholesterol to the denatured DNA makes optical density increase by 37% compared to control (Fig. 1).

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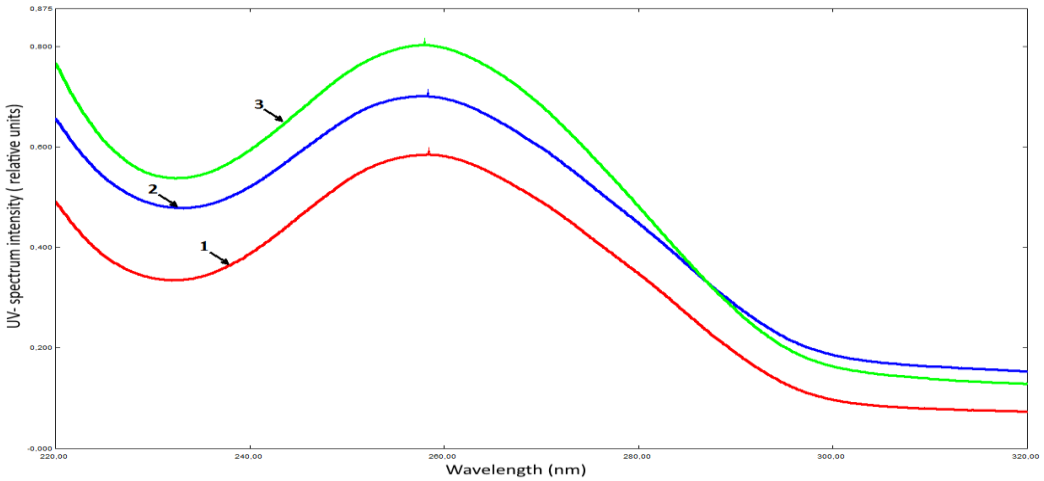

Figure 1 – Absorption spectra of DNA isolated from donors blood under the influence of cholesterol (1 – control, 2 – DNA+cholesterol, 3 – denaturated DNA + cholesterol)

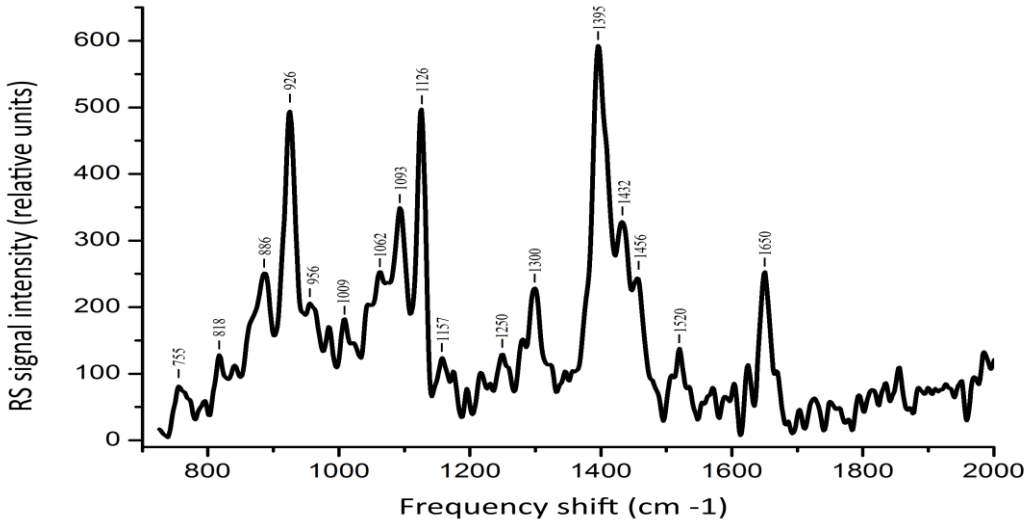

Figure 2 – DNA RS spectra

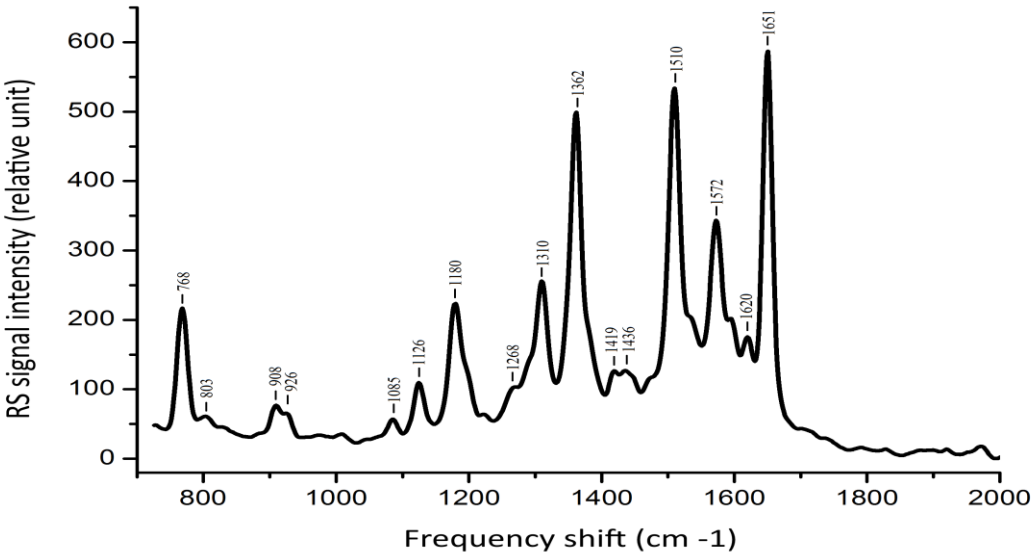

Figure 3 – DNA RS spectra under the influence of cholesterol

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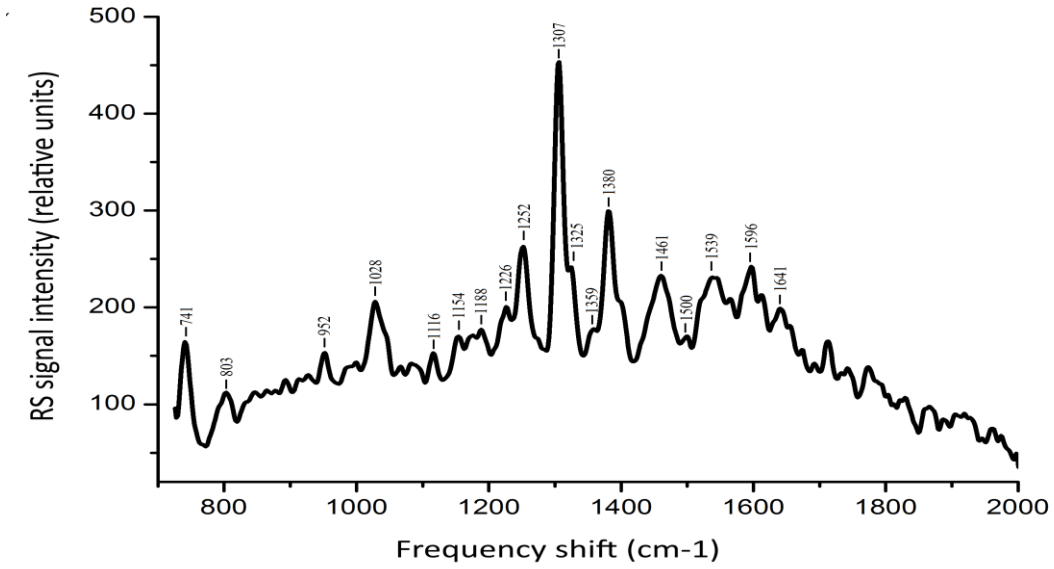

Figure 4 – DNA RS spectra of the DNA-cholesterol complex under the influence of MBC

Therefore, the formation of the cholesterol-DNA complex accompanies the hyperchromic effect, and apparently lipid-DNA interaction efficiency increases with unwinding (melting) of the nucleic acid double helix. This fact of improved affinity of cholesterol to single-stranded DNA can occur during transcription, when there is a partial melting of the DNA strands, and apparently, it has a functional significance, since the increase of hydrophobicity in certain DNA loci can affect the efficiency of binding to the oligonucleotide sequences of different proteins.

To study the conformational features of cholesterol-DNA complex by Raman spectroscopy method, certain Raman spectrum bands in the frequency shift range were used [9].

The Raman spectrum of the DNA extracted from nucleated cells of donors' blood is characterised by the presence of absorption bands associated with fluctuations of the deoxyribose (926, 1432 cm^{-1}), metaphosphate O - P - O (1093 cm^{-1}) ; deoxyribose phosphate (1126 cm^{-1}); uracil (1250 cm^{-1}); adenine (1300, 1520 cm^{-1}), T, A, G - AG (1395 cm^{-1}); C = O bond (1662 cm^{-1}).

Under the influence of cholesterol an offset of bands occurs in the DNA RS with respect to basic bonds and new bonds emerge (Figures 3-5). Several bands associated with vibrations of connection C-N-1180, G, A - 1572 cm^{-1} appear. Furthermore, there is a displacement and "damping" of the bands 803 and 1085 cm^{-1}, associated with fluctuations of metaphosphate O - P - O (818 and 1094 cm^{-1} respectively).

Therefore, cholesterol leads to a change in the conformation of groups of atoms within the DNA helix as well as in the sugar-phosphate backbone facing outwardly of the double helix. Influenced by cholesterol, excitations of the nitrogen bases atoms intensify, which confirms the above mentioned fact of weakening hydrogen bonds in the double-stranded DNA molecules under the influence of lipid and, as a consequence, facilitating the unwinding of the DNA double helix.

With methyl-beta-cyclodextrin (MBC), which has a high affinity for cholesterol [8], it is shown that adding of MBC "smoothes" some effects of cholesterol on the conformation of DNA. The Raman spectrum of cholesterol-DNA complex, when treated with methyl-beta-cyclodextrin, there are bands of 952 cm^{-1} (956 cm^{-1} - deoxyribose), 1028 cm^{-1} (1017 cm^{-1} - C = O bond); 1154 cm^{-1} (1157 cm^{-1} - deoxyribose phosphate), bands related to A are very prominent - 1305 cm^{-1}; T, A, G - 1380 cm^{-1}.

Methyl-beta-cyclodextrin has a high cholesterol-solubilizing ability and it "pulls" the cholesterol associated with DNA thus significantly weakening the effects provided by cholesterol, detected in the analysis of the corresponding Raman spectra.

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CONCLUSIONS

Cholesterol may bind itself with varying degrees of specificity with specific DNA sequences. Actually, compared to other lipids, cholesterol is present in DNA in greater numbers. Placed in the minor groove of DNA, cholesterol may influence the conformation of biomolecules, and efficiency of interaction of DNA with protein transcription factors. Cholesterol can be regarded as an additional element of the protein regulation of gene expression, as it influences and changes the conformation of DNA and probably its ability to interact with transcriptional protein factors.

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