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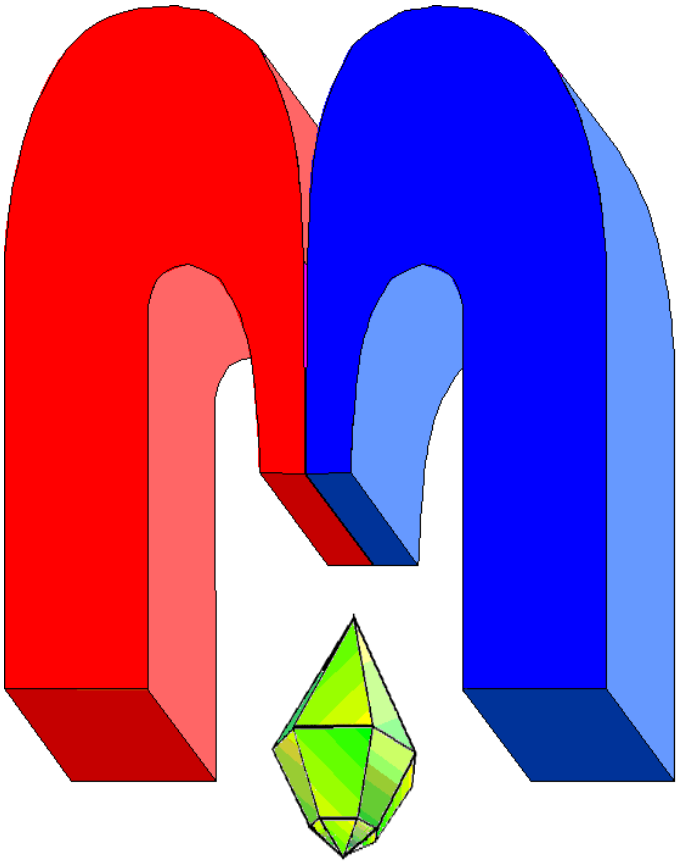

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* In Kazan University the Electron Paramagnetic Resonance (EPR) was discovered by Zavoisky E.K. in 1944.

Investigation of «cholesterol + model of biological membrane» complex by NMR spectroscopy†

L.F. Galiullina*, D.S. Blokhin, A.V. Aganov, V.V. Klochkov
Kazan Federal University, Kremlevskaya, 18, Kazan 420008, Russia
**E-mail: lgaliull@ksu.ru*

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On the basis of the nuclear magnetic resonance (NMR) experiments it was established that molecules of sodium dodecyl sulfate can form micelles in dimethyl sulfoxide solution. The nuclear Overhauser effect between OH-group of cholesterol and "tail" groups of sodium dodecyl sulfate hydrophobic part was observed in 1D selective NOESY experiment. This observation corresponds to close spatial arrangement of these parts of different molecules and the presence of a complex between cholesterol and sodium dodecyl sulfate micelles.

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Keywords: nuclear magnetic resonance spectroscopy, cholesterol, sodium dodecyl sulfate, micelles, nuclear Overhauser effect.

1. Introduction

Cholesterol is one of the basic components in cell membranes. It regulates the membrane permeability and the membrane enzymes activity. Cholesterol is also responsible for a cell survival and new cells formation in their division process. Thus cholesterol plays a crucial role in the cells biosynthesis and metabolism [1], and generally in an organism vital functions. Quantity of the cholesterol in its different fractions is considered as the risk factor in the atherosclerosis evolution (the cholesterol theory of atherosclerosis), other cardio - vascular system diseases and the Alzheimer's disease [2, 3]. Cholesterol molecules are hydrophobic and form the different molecular complexes with acids, proteins, amines and salts. This fact probably causes difficulties in establishing cause-and-effect relationships in the study of the effect of cholesterol on the organism.

The capabilities of modern nuclear magnetic resonance (NMR) spectroscopy techniques to study the structure and intermolecular interactions in the phospholipid membranes are still very limited. NMR technique using nuclear Overhauser effect is the most effective method for such investigations. However, there is a problem in using this technique for the phospholipid membrane studies because the proton relaxation time of the phospholipid aggregates is short relative to the NMR time-scale. In addition, cholesterol is poorly soluble in the water, and most of the phospholipids are not soluble in the organic solvents. Therefore, the actual problem is finding the appropriate model systems for investigation by high-resolution NMR spectroscopy.

In contrast to the phospholipids, which form bilayers and multilayers in the aqueous solutions, sodium dodecyl sulfate (SDS) molecules can form a micelles in solution. Micelles are relatively small spherical aggregates distributed throughout the solution. Hence, SDS is a suitable model system for studying interactions between the various components of cell membranes, particularly cholesterol, and phospholipid membranes [4-6].

The purpose of this work is to investigate the structure and properties of the cholesterol - sodium dodecyl sulfate intermolecular complex in solution using high-resolution NMR spectroscopy modern techniques.

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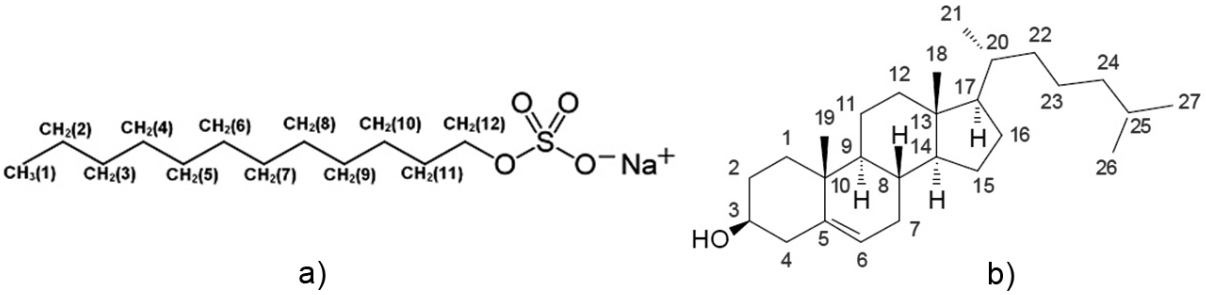

Figure 1. The chemical structures of sodium dodecyl sulfate (a) and cholesterol (b).

2. Materials and methods

^{1}H NMR (500.13 MHz) and ^{13}C NMR (125.758 MHz) spectra were recorded on an "Avance II-500" spectrometer (Bruker). The spectrometer operates in the internal ^{2}H resonance stabilization. All spectra were acquired in a 5-mm inverse probehead in 5-mm tubes at temperature 298 (±0.1) K. Samples for the experiments were solutions of cholesterol (concentration 2 g/l), SDS (concentration varied from 2 g/l to 80 g/l) and cholesterol+SDS mixture (cholesterol concentration 2 g/l, SDS concentration 80 g/l) in mixed solvent DMSO+H_2O (5:1). Sample volume is 500 µl. Chemical shifts (ppm) are internally referenced to the signal of DMSO methoxyl groups (δ ^{1}H 2.5 ppm, δ ^{13}C 39.5 ppm) in all cases. ^{1}H NMR spectra were recorded using 90° pulses with duration of 7.0 µs and the delay between pulses of 2 s, with the spectral width of sw = 9.40 ppm. ^{13}C NMR spectra were recorded using 45° pulses and broadband decoupling from protons. The pulse duration was 14.0 µs, the delay between pulses was 2 s and the spectral width was 200 ppm. Complete assignments of the ^{1}H and ^{13}C NMR spectra of the title compounds were accomplished by 2D COSY, HSQC and HMBC experiments. Accuracy of ^{1}H and ^{13}C NMR chemical shifts measurement was ± 0.01 ppm and ± 0.1 ppm respectively. Nuclear Overhauser effects measurement were carried out be selective 1D NOESY experiments [7, 8]. For radiofrequency irradiation Gauss-shaped pulses were used. The repetition time between subsequent runs of the NOESY sequence was at least three times longer than the mean decapeptide proton T_1 for SDS and cholesterol molecules. Mixing time values, τ_m, were 0.05, 0.1, 0.15, 0.2, 0.4, 0.6 и 0.8 s.

3. Results and discussion

Sodium dodecyl sulfate micelle formation in DMSO+H_2O solution by NMR NOESY spectroscopy data

The initial step of this study was the recording and analysing ^{1}H NMR spectra of SDS (fig. 1,a) in DMSO+H_2O solution at concentrations below (2 g/l) (fig. 2) and above (80 g/l) (fig. 3) the critical micelle concentration [9].

The signals of the methyl group CH_3-1, methylene groups CH_2-12 and CH_2-11 are resolved in ^{1}H NMR spectra of SDS (fig. 2,a and fig. 3,a). The signals of other functional groups of SDS (CH_2-(2-10)) are represented in the spectrum as one intensive broad signal in 1.2-1.25 ppm region. It can be seen that the chemical shifts of the signals in the spectrum with a higher concentration (80 g/l, fig. 3,a) differ from the chemical shifts of the signals in solution with a lower concentration (2 g/l, fig. 2,a). In particular, with the concentration increasing low-field shift of CH_2-12 signal is observed (+0.7 ppm). At the same time the signals of CH_2-(2-10) and CH_3-1 are slightly high-field shifted (−0.1 ppm). Such changes in chemical shifts indicate the appearance of intermolecular complexes, which are SDS micelles.

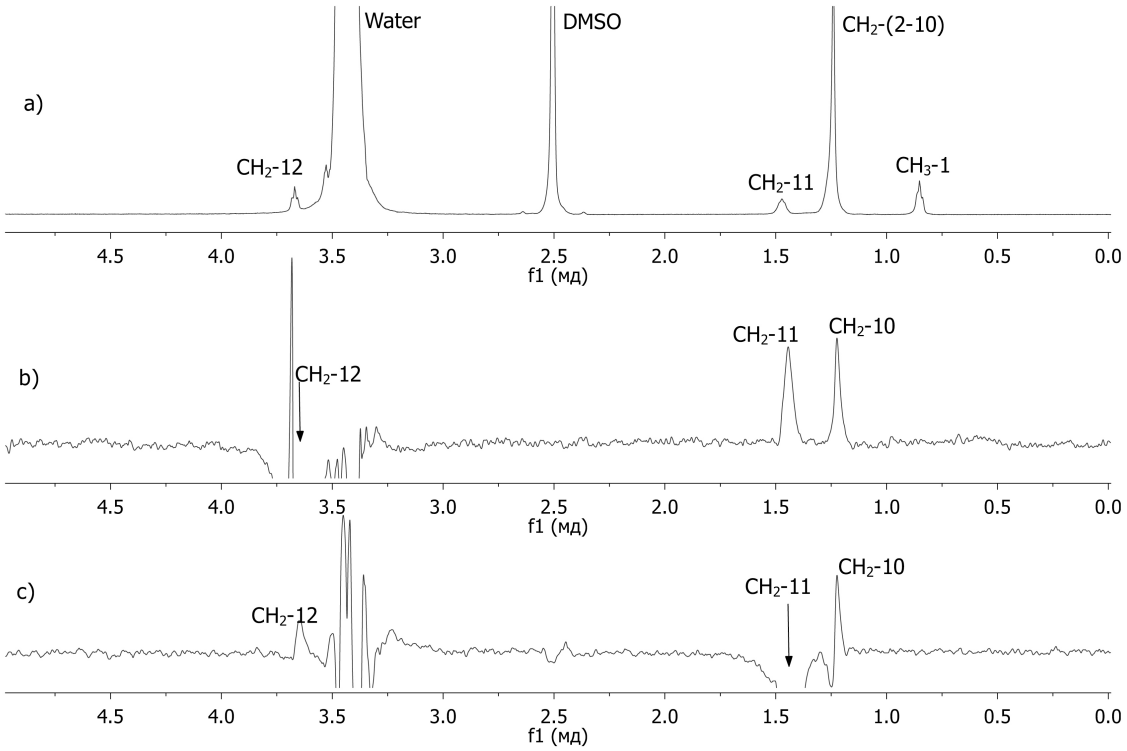

Figure 2. ^{1}H NMR (500.13 MHz) spectrum (a) and selective 1D NOESY spectra (b, c) of SDS in DMSO+H_2O solution at T = 298 K (concentration 2 g/l). The arrow shows the signal with the frequency of applied selective RF pulse. Mixing time is $\tau_м$ = 400 ms.

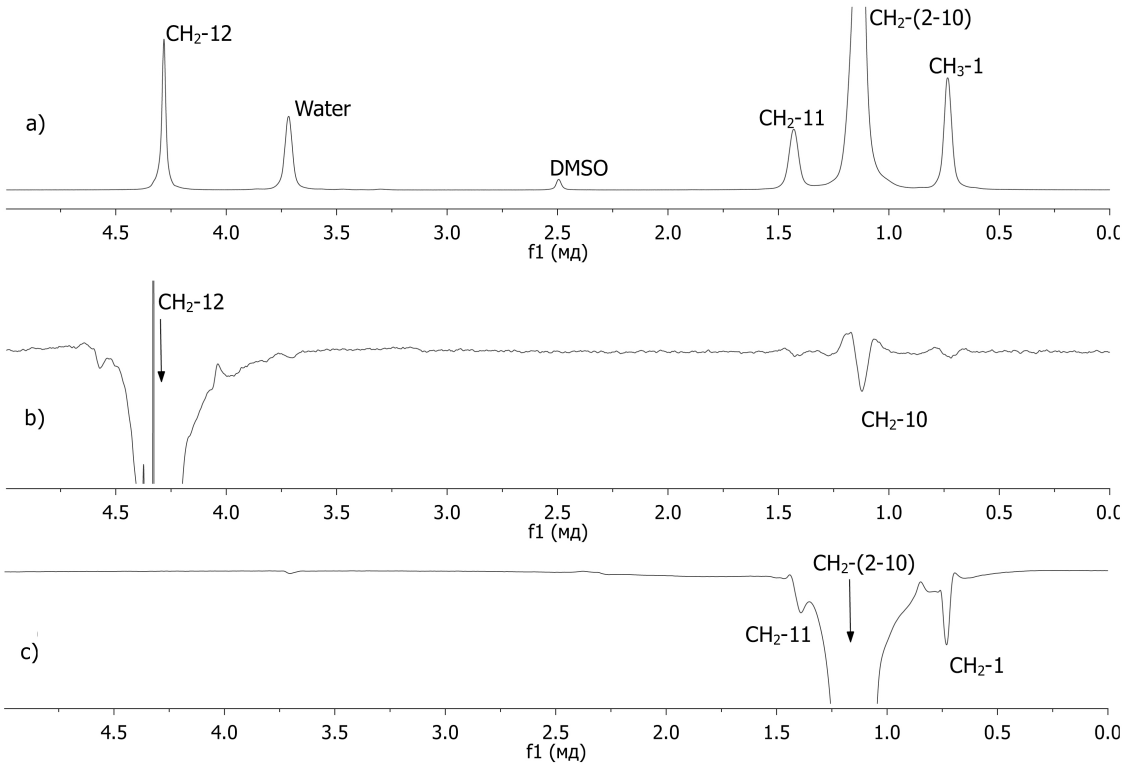

Figure 3. ^{1}H NMR (500.13 MHz) spectrum (c) and selective 1D NOESY spectra (a, b) of SDS in DMSO+H_2O solution at T = 298 K (concentration 80 g/l). The arrow shows the signal with the frequency of applied selective RF pulse. Mixing time is $\tau_м$ = 50 ms.

NOESY method is an effective technique to study formation of different intermolecular complexes. It is known that the maximum of observed Overhauser effect depends on the correlation time of molecular motion which is related to the size of molecules [10-13]. Overhauser effect is positive for small molecules (molecular mass < 600) and negative for large molecules (molecular mass > 1200) [14]. Thereby, expected Overhauser effect for the monomeric form of SDS should be positive, and for the micellar form – negative.

At low concentrations of SDS (2 g/l) (fig. 2) in NMR selective 1D NOESY spectra of sample irradiated at signal frequency of CH_2-12 group is observed Overhauser effects for the atomic groups CH_2-11 and one of the groups that contribute to the intense signal of CH_2-(2-10). Most likely this is the group CH_2-10, which is closest to the irradiated CH_2-12 group.

In 1D NOESY NMR spectrum of the sample irradiated on CH_2-11 signal frequency the Overhauser effect is observed for CH_2-10 and CH_2-12 signals. All of the observed Overhauser effects are positive, and thus correspond to small molecules. It means that SDS at this concentration is in a monomeric form. At high concentrations of SDS (80 g/l) (fig. 3) in 1D selective NOESY NMR spectra of the sample irradiated on frequency of CH_2-12 group Overhauser effect is observed for CH_2-10 signal. If the sample irradiated on the frequency of intensive signal from CH_2-(2-10) groups, negative Overhauser effect is observed for CH_3-1 and CH_2-11 groups.

The negative Overhauser effect is typical for the large molecules. Therefore, SDS in the solution is presented in a micellar form. Dimethylsulfoxide (DMSO) is an aprotic organic solvent. Hence, SDS molecules can form reverse type micelles in the DMSO solution [15].

Cholesterol – sodium dodecyl sulfate complex formation in DMSO+H_2O solution by NMR spectroscopy data

The chemical shifts of SDS (fig. 4,c) and cholesterol (fig. 1,b) signals (fig. 4,b) in DMSO+H_2O solution separately are almost the same as chemical shifts of those signals in the spectrum of SDS+cholesterol mixture (fig. 4,a). Small differences are observed for the most closest to OH-group atoms of cyclic part of cholesterol. Therefore, if there is an interaction between cholesterol molecules and SDS micelles in solution, then it involves OH-group of cholesterol molecule.

The presence of a molecular complex between cholesterol and SDS micelles was established using one-dimensional selective NOESY experiments (fig. 5) [7, 8].

In 1D NOESY NMR spectrum of the sample irradiated on OH-group of cholesterol signal frequency the Overhauser effect is observed for signals of water, CH_3-1 group of SDS and for the one of aliphatic chain CH_2 groups of SDS molecule. Most likely, it is a signal from the CH_2-2 group of SDS, which is the nearest to the methyl group CH_3-1 of SDS. It means that the dipole-dipole interaction between OH-group of cholesterol and “tail” groups of SDS hydrophobic part (fig. 5). This indicates a spatial proximity of these molecules and the presence of a complex between cholesterol and SDS micelles. Schematic presentation of the complex is shown on fig. 6.

This observation is in agreement with the real molecular systems. In the interaction of cholesterol with phospholipid membranes, it penetrates into the space between the hydrophobic “tails” of phospholipid bilayers [16, 17]. Thus, the proximity of cholesterol OH-group and “tail” aliphatic groups of SDS is similar to the proximity of the same groups of atoms of cholesterol and aliphatic chains of phospholipid molecules. The only difference is that in phospholipid membranes cholesterol penetrates “deeper” into the space between the hydrophobic “tails” of the phospholipid molecules, but in the model system cholesterol interacts almost exclusively with the surface of the micelles SDS.

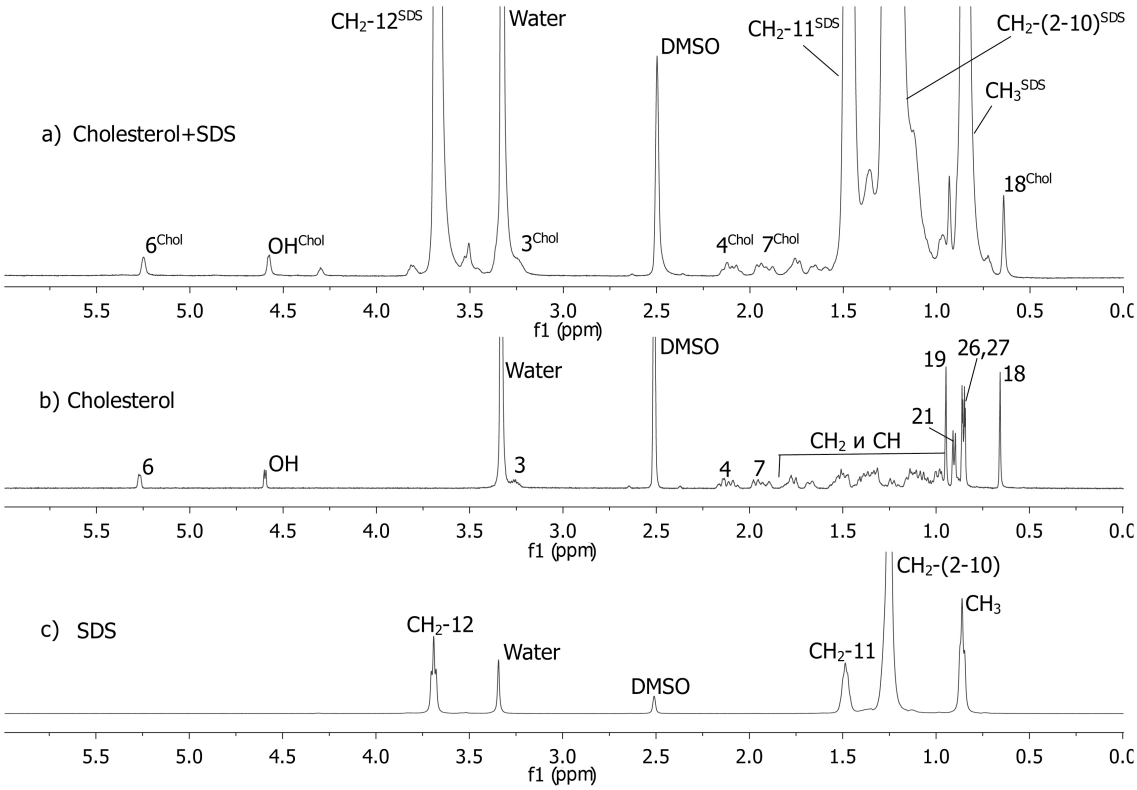

Figure 4. ^{1}H NMR (500.13 MHz) spectra of SDS (c), cholesterol (b) and cholesterol+SDS mixture (a) in DMSO+H_2O solution at T = 298 K. Chemical structures of cholesterol and SDS molecules with atom numeration are also shown on the figure.

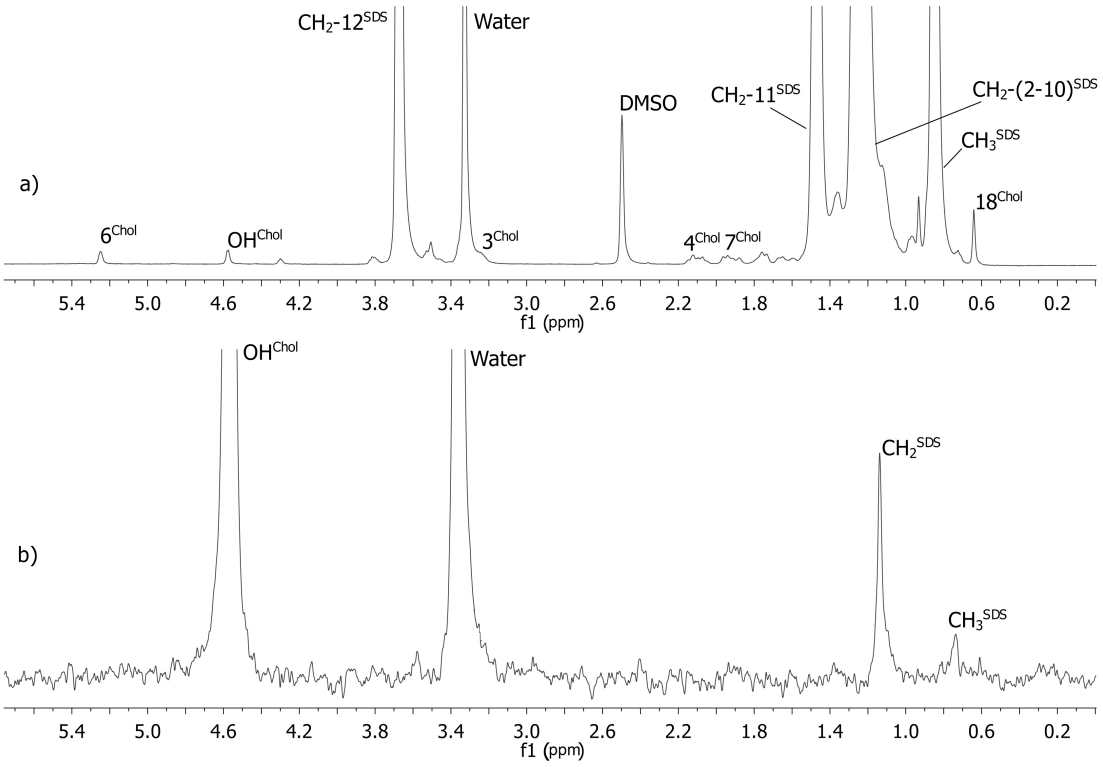

Figure 5. Selective 1D NOESY spectrum (b) and ^{1}H NMR (500.13 MHz) spectrum (a) of cholesterol+SDS mixture in DMSO+H_2O solution at T = 298 K. Mixing time is $\tau_м$ = 50 ms. The chemical structures of cholesterol and SDS molecules with atom numeration are also shown on the figure. The arrow shows the atoms for which Overhauser effect is observed.

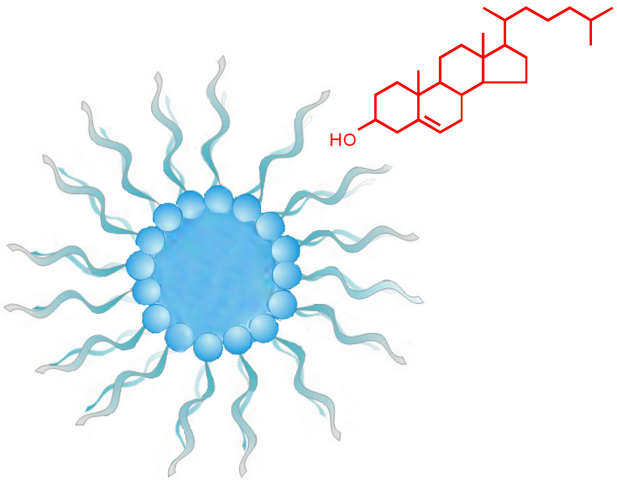

Figure 6. Schematic presentation of the complex between cholesterol and SDS micelles.

4. Summary

The results of NMR experiments showed that sodium dodecyl sulfate aggregates into reversed micelles in DMSO+H_2O solution at concentrations higher than critical micelle concentration. Cholesterol molecules form intermolecular complex with sodium dodecyl sulfate micelles by interaction of the OH group of cholesterol with CH_3-1 and CH_2-2 "tail" aliphatic groups of sodium dodecyl sulfate molecule. This interaction is similar to cholesterol behavior in the phospholipid bilayer membranes, where cholesterol penetrates with its cyclic part into space between the hydrophobic "tails" of the phospholipid molecules oriented mostly orthogonal to bilayers.

Acknowledgments

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