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Structure of pravastatin and its complex with sodium dodecyl sulfate micelles studied by NMR spectroscopy

L. F. Galiullina,* I. Z. Rakhmatullin, E. A. Klochkova, A. V. Aganov and V. V. Klochkov

The aim of this work was to study the mechanisms of interaction between pravastatin and cell membranes using model membranes (sodium dodecyl sulfate micelles) by nuclear magnetic resonance spectroscopy methods. On the basis of the nuclear magnetic resonance experiments, it was established that pravastatin can form intermolecular complexes with sodium dodecyl sulfate micelles by the interaction of its hydrophilic groups with the polar surface of the micelle. Conformational features of pravastatin molecule were also studied. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: NMR spectroscopy; complexation; pravastatin; sodium dodecyl sulfate; nuclear overhauser effect; biophysical models; surfactants; micelle

Introduction

Statins, such as pravastatin, play a crucial role in the management of hypercholesterolemia. They are well-known potent 3-hydroxy-8-methylglutaryl CoA (HMG-CoA) reductase inhibitors. These compounds also have beneficial actions in many other pathological conditions, such as osteoporosis and osteoporosis-related bone fractures, cardiac diseases, and neurological disorders.[1] To understand the biochemical basis of such important properties of statins, it is necessary to study their physicochemical characteristics and pharmacology at the molecular level.

The efficacy, metabolism, and safety of statins depend on their location in molecular membranes.[2] An understanding of how these drugs interact with cellular membranes may further help to elucidate an origin of their pharmacologic properties.

Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for a detailed study of the biophysical properties of medical objects such as statins.[3–9] Particularly, NMR techniques using nuclear Overhauser effects (NOEs) are one of the most effective methods for investigations of conformational structures of statins and their intermolecular complexes with different compounds.[1,10–12] However, the capabilities of modern NMR spectroscopy techniques to study the structure and intermolecular interactions in phospholipid membranes are still very limited. There is a problem in using this technique for studies of interactions in phospholipid membranes because T_2 proton relaxation times of phospholipid aggregates are short relative to the NMR chemical shift timescale. Nevertheless, interactions of different drugs with phospholipid aggregates can be effectively studied by NMR using model membranes. One of the commonly used membrane models in NMR work is the sodium dodecyl sulfate (SDS) micelle.[13–19] The detergent head groups of SDS may be designed to physically mimic the surface of a biological membrane.

The aim of this work was to study the mechanisms of interaction between pravastatin and cell membranes using model membranes by NMR spectroscopy methods. The results presented in this paper may help to understand the origin of the pharmacological and physicochemical properties of different statins, in particular, of pravastatin.

Experimental section

Pravastatin was purchased from Aldrich and used without further purification. Pravastatin samples were dissolved in D_2O with concentrations of 6 g/l. The concentration of SDS in D_2O solution was greater than the critical micelle concentration (8.2 mM), diameter of micelle – 5 nm. Solution volume was 0.6 ml, pH = 6.0.

All NMR experiments were performed on a Bruker Avance II-500 NMR spectrometer equipped with a 5 mm probe using standard Bruker TOPSPIN software. Temperature control was performed using a Bruker variable temperature unit (BVT-2000) in combination with a Bruker cooling unit (BCU-05) to provide chilled air. Experiments were performed at 303 K without sample spinning. Chemical shifts are given in values of parts per million (ppm), referenced to residual D_2O solvent signals (4.70 for ^1H). ^1H NMR data were collected with 32 k complex data points and were apodized with a Gaussian window function (lb = −0.5 and gb = 0.2) prior to Fourier transformation. Signal-to-noise enhancement was achieved by multiplication of the free induction decay with an exponential window function (lb = 1 Hz).

Assignments of ^1H and ^{13}C NMR signals of pravastatin dissolved in D_2O were achieved from signal multiplicities, integral values,

* Correspondence to: L. Galiullina, Institute of physics, Kazan Federal University, 420008 Kazan, Russia. E-mail: lgaliull@kpfu.ru

Institute of Physics, Kazan Federal University, 420008 Kazan, Russia

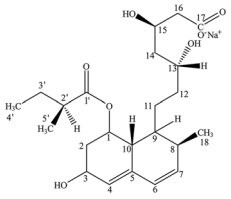

Figure 1. Chemical structure of pravastatin.

andcharacteristic chemical shifts from the through-bond correlations in 2D COSY spectra, through-space correlations in 2D NOE spectroscopy (NOESY) spectra, and ^{1}H-^{13}C heteronuclear correlations in 2D heteronuclear single-quantum correlation (HSQC) and HMBC spectra.

All 2D experiments were performed with 2 k × 512 data points; the number of transients (96 scans) and the sweep widths were optimized individually. In the homonuclear ^{1}H,

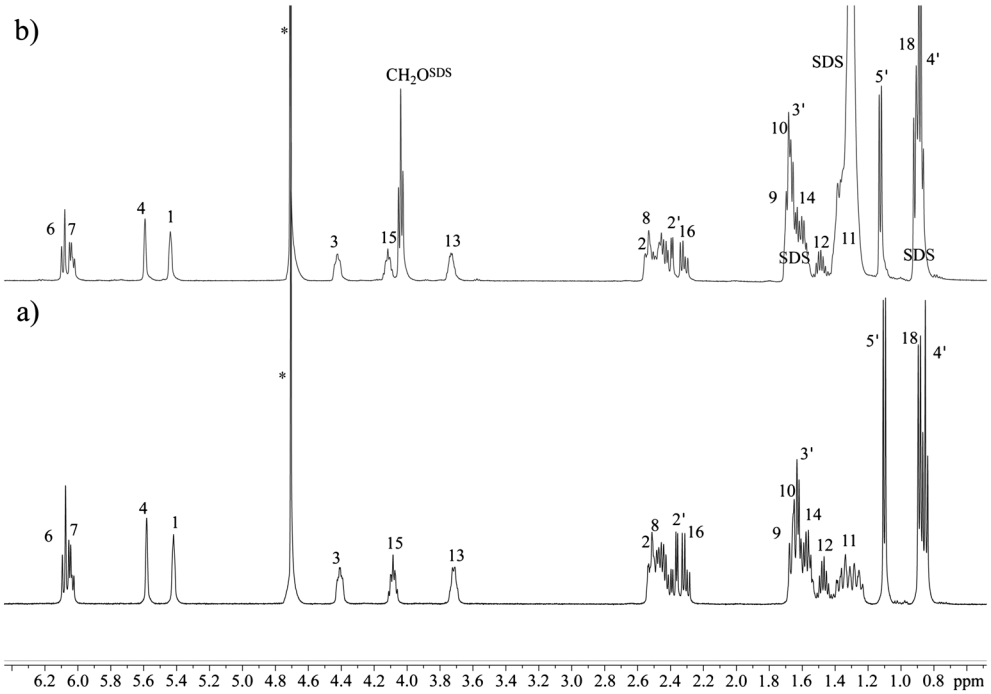

Figure 2. ^{1}H NMR spectra of the (a) pravastatin and (b) pravastatin + sodium dodecyl sulfate (SDS) micelles in D_2O at T = 303 K. The signal of $\underline{H}_2O$ is marked by *. Some groups of SDS are not signed for better readability of the figure.

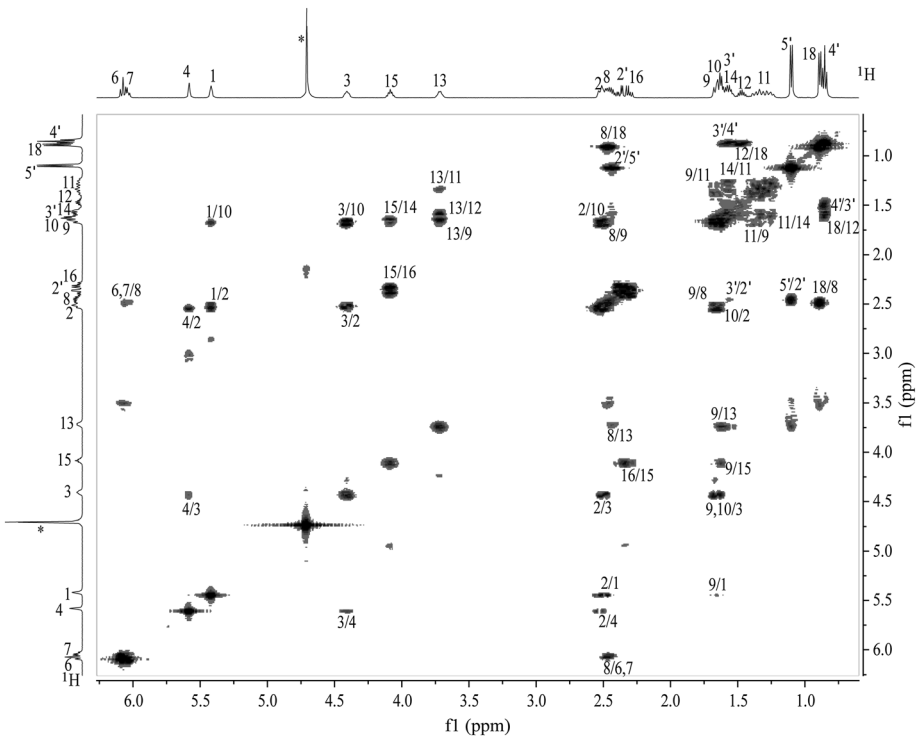

Figure 3. ^{1}H-^{1}H COSY NMR spectrum of the pravastatin in D_2O at T = 303 K.

^{1}H COSY (Bruker pulse program *cosygpqf*), and 2D ge-NOESY,[20] experiments were performed with pulsed filtered gradient techniques;[21] the relaxation delay was set to 2 s and the 90° pulse length to 7.5 μs. Mixing time values were 0.50, 0.20, 0.15, 0.10, and 0.05 s. The resulting free induction decays were zero-filled to a 2 k × 1 k data matrix and apodized with a sine function for COSY and a shifted sine function for NOESY in both the ω1 and ω2 dimensions prior to Fourier transformation. Heteronuclear spectra were recorded with 2 k × 512 data points, zero-filled in F1 to a 2 k × 512 data matrix, and apodized in both dimensions with a shifted sine function. HSQC experiments (*hsqcetgpsp*) were acquired using adiabatic pulses for inversion of ^{13}C and globally optimized alternating phase rectangular pulse sequence for broadband ^{13}C-decoupling, optimized for ^{1}J(CH) = 145 Hz. ^{1}H-^{13}C long-range spectra HMBC (*hmbcgplpndqf*) were performed with ^{n}J(CH) set to 8 Hz.

Results and discussion

It is known that in contrast to most statins, pravastatin dissolves readily in water. This fact gave us the opportunity for recording NMR spectra of pravastatin in the most common biologically native liquid.

The chemical structure of pravastatin (Fig. 1), dissolved in D_2O, was confirmed by the results of 1D ^{1}H and ^{13}C and 2D NMR experiments. The signals in ^{1}H NMR spectrum (Fig. 2(a)) were assigned using 2D NMR techniques, including ^{1}H-^{1}H COSY (Fig. 3), ^{1}H-^{13}C HSQC, and ^{1}H-^{13}C HMBC spectra (Supporting Information).

The signals of methyl protons CH_3-5′, 18, and 4′ are observed in the high-field region at 1.10, 0.89, and 0.85 ppm, respectively, in the ^{1}H NMR spectrum of pravastatin (Fig. 2(a), Table 1). The protons CH-6,7,4 resonate in the downfield region at 6.08, 6.04, and 5.58 ppm, respectively. The signals of protons CH-1,3,15,13 are also well resolved in the spectrum (Fig. 2(a)). There are a number of overlapping signals in the ranges of chemical shifts δ = 1.20–1.70 and 2.20–2.60 ppm, which are assigned in Fig. 2. The signals of OH groups do not appear in the ^{1}H NMR spectrum because they are involved in a fast exchange relative to the NMR chemical shift time-scale exchange with protons of H_2O. The 2D ^{1}H-^{1}H COSY spectrum (Fig. 3) clearly reveals all of the nearby protons of the compound.

To define the conformational structure of the pravastatin molecule in solution, 2D NOESY NMR experiments were performed. There are several nontrivial cross peaks observed in the spectrum (Fig. 4). Cross peaks between the protons of the

Table 1. ^{1}H NMR chemical shifts (δ, ppm) of pravastatin in (a) D_2O and in (b) D_2O + sodium dodecyl sulfate (SDS) at 303 K

	CH_3-4′	CH_3-18	CH_3-5′	CH_2-11	CH_2-12	CH_2-14	CH-10	CH_2-3′
(a)	0.85	0.89	1.10	1.29	1.35	1.57	1.65	1.47; 1.63
(b)	0.87	0.91	1.12	1.29	1.35	1.60	1.67	1.48; 1.63
	CH-9	CH_2-16	CH-2′	CH-8	CH_2-2	CH-13	CH-15	CH_2O^{SDS}
(a)	1.68	2.31	2.36	2.46	2.52	3.72	4.09	—
(b)	1.69	2.32	2.38	2.46	2.54	3.73	4.11	4.03
	CH-3	CH-1	CH-4	CH-7	CH-6			
(a)	4.41	5.42	5.58	6.04	6.09			
(b)	4.42	5.43	5.59	6.03	6.08			

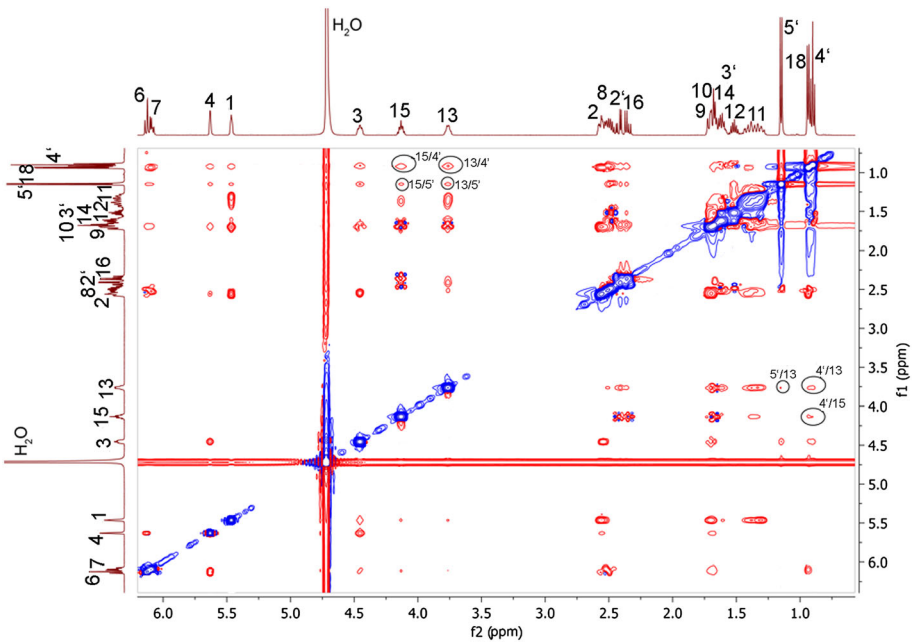

Figure 4. 2D nuclear Overhauser effect spectroscopy NMR spectrum of pravastatin in D_2O solution at T = 303 K. Mixing time is τ_M = 200 ms.

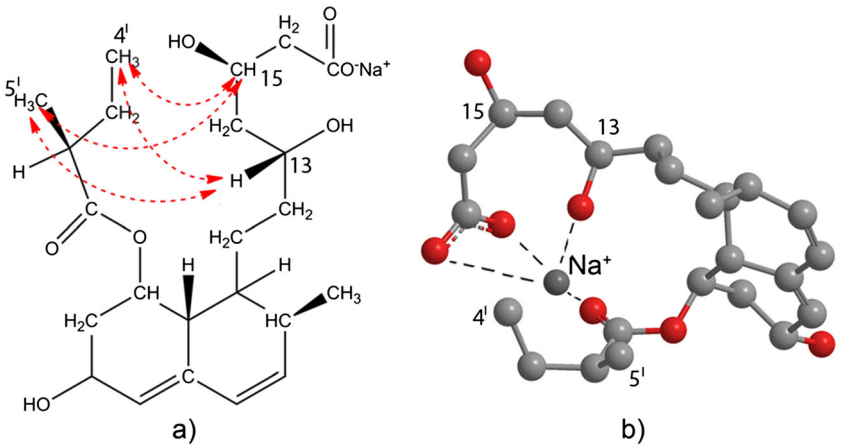

Figure 5. Schematic presentation of the (a) observed nuclear Overhauser effect (dashed lines) and (b) conformational structure (hydrogen atoms are not shown) of pravastatin.

aliphatic chain CH-13 and CH-15 and methyl protons CH_3-4′ and CH_3-5′ (Figs 4 and 5) provide the information about conformational features of the pravastatin molecule.

Observed NOEs indicate the proximity of two aliphatic chains of the molecule (Fig. 5). This can be explained by the interaction of partially negatively charged oxygen atoms of both chains with positively charged Na^+ ion as it schematically shown in Fig. 5.

Study of the intermolecular complex formed by pravastatin and model membranes (SDS micelles) can be useful for understanding of the basic principles of interaction between pravastatin and cell membranes. ^{1}H NMR spectrum of pravastatin dissolved in D_2O solution with SDS micelles is shown in Fig. 2(b). Adding of the SDS micelles leads to changes in chemical shifts of some signals (Table 1). In particular, small low-field shifts (+0.01 and +0.02 ppm) are observed for the signals of the groups close to hydrophilic parts of the molecule (i.e., CH-2′,1,3,4,13; CH_2-16; CH_3-5′). The signals CH-6,7 experience slight low-field shifts (−0.01 ppm). In contrast, the chemical shifts of the lines related to the protons CH-8 and CH_2-11,12 are not changed. This indicates that in D_2O solution with SDS micelles, pravastatin molecules take part in some additional intermolecular interaction and possibly form complexes with the molecules of SDS.

In order to investigate the mechanisms of complex formation between pravastatin and SDS micelles, 2D NOESY experiments

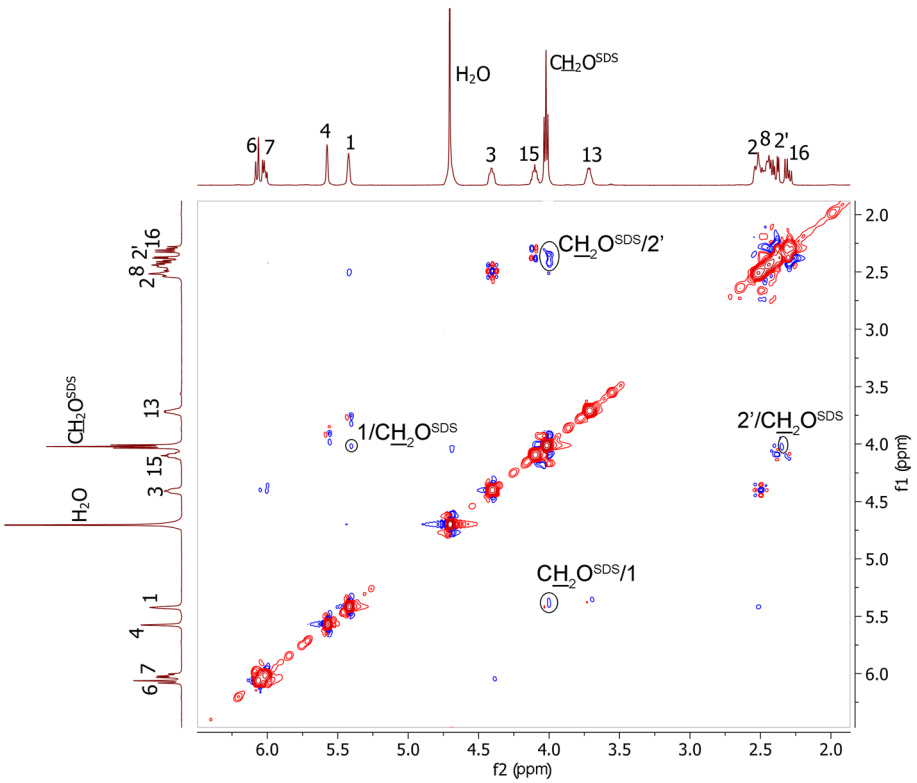

Figure 6. Fragment of 2D nuclear Overhauser effect spectroscopy NMR spectrum of pravastatin + sodium dodecyl sulfate mixture in D_2O solution at T = 303 K. Mixing time is τ_M = 50 ms.

were carried out (Fig. 6). The cross peaks between CH-1 and CH_2-2′ groups of pravastatin and CH_2O protons of the 'head' group of SDS are observed in the spectrum. Another NOE between pravastatin and SDS molecules is not presented in Fig. 6 because they are overlapped with noise that appeared from intensive SDS signals. Nevertheless, it can be concluded that pravastatin forms an intermolecular complex with SDS micelles by the interaction of oxygen atoms of pravastatin with polar head of SDS, as could be expected.

It means that pravastatin is located on the polar surface of the micelles that are used as model membranes. This conclusion does not contradict the known observations of Mason *et al*.[2] that pravastatin is restricted to the hydrated, polar surface of the cell membrane. Thus, SDS micelles can be used as model membranes for investigations of interaction between pravastatin and cell membranes.

In fact, pravastatin cannot passively penetrate the cell as other, more lipophilic statins can, but it requires an active transport system.[22] Results obtained in this work also showed that pravastatin does not penetrate to the model membrane (SDS micelle) and interacts only with its surface.

Conclusions

The results of 2D NOESY NMR experiments showed that pravastatin can form an intermolecular complex with SDS micelles by the interaction of its hydrophilic groups with the polar surface of the micelle. Conformational features of the pravastatin molecule were also studied. It was shown that two aliphatic chains of the pravastatin molecule are closely located to each other because of the interaction of partially negatively charged oxygen atoms of both chains with a positively charged Na^+ ion.

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