International Journal of Biomolecules and Biomedicine (IJBB)
ISSN: 2221-1063 (Print) 2222-503X (Online)
Vol. 4, No. 1, p. 1-6, 2014
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Interaction of chenodeoxycholic acid with cholesterol in a model system studied by spin label probe method

Sh. A. Kholova[1], Kh. Sh. Dzhuraev[1], I. Kh. Yusupov[2], G. I. Likhtenshtein[3*]

[1]*The State Research Institute of Nourishment, Dushanbe, Tadzhikistan Republic*

[2]*The S.U. Umarov Physico-Technical Institute, Academy of Science of Tadzhikistan Republic, Dushanbe, Tadzhikistan Republic*

[3]*Ben-Gurion University of the Negev, Beer-Sheva, Israel*

Article Published: January 03, 2014

Key words: Cholesterol, chenodeoxycholic acid, gallstones, nitroxide spin probe, electron spin resonance.

Abstract

Interaction of cholesterol which is an essential structural component of animal cell membranes that is required to establish proper membrane permeability and fluidity and the bile chenodeoxycholic acid which significantly increased serum total cholesterol, and high-density-lipoprotein (HDL) cholesterol, and dissolves gallstones appears to be one of pivotal problem of biomedical gastroenterology. The methods of spin labels and spin probes have been proved to be power tools for solving structural and dynamical problems of chemistry and biology on molecular level. In present work the method of spin probes was used for investigation of quantitative interaction of cholesterol and chenodeoxycholic acid in ethanol possessing dielectric properties similar to ones of biological membranes. Using Electron Spin Resonance of nitroxide spin probe, it was shown that in the ethanol solution cholesterol molecules form aggregates, modeling a gallstone, which effectively bound chenodeoxycholic acid. The developed method can be used for study relationships between cholesterol and chenodeoxycholic acid in the in human blood, high-density-lipoproteins and gallstones.

*Corresponding Author: G. I. Likhtenshtein ✉ gertz@bgu.ac.il

Introduction

Relationship between cholesterol and chenodeoxycholic acid CDOCA appears to be one of pivotal problem of biomedical gastroenterology. Cholesterol is an essential structural component of animal cell membranes that is required to establish proper membrane permeability and fluidity (Ohvo-Rekilä et al., 2002, Incardona and Eaton S. 2000, Beers *et al.,* 2006). Cholesterol gallstones, which are a crystalline structure formed within the gallbladder by accretion of bile components, are among the most common gastrointestinal disorders. Individuals with gallstones may experience various gastrointestinal symptoms and are also at risk of developing acute or chronic cholecystitis (Alan and Gaby, 2009).

Chenodeoxycholic acid is a bile acid naturally found in the body. Chenodeoxycholic acid is one of the most important human bile acids. CDOCA suppressed bile acid synthesis, significantly increased serum total cholesterol, and significantly increased high-density-lipoprotein (HDL) cholesterol. (Fromm, 1989).

When administered in pharmacological doses it causes a decrease in cholesterol saturation of bile, which in turn may lead to gradual dissolution of cholesterol gallstone. (Iser and Sali, 1981, Doty *et al.,* 1982, Petroni et al. 2001; Marschall and Einarsson, 2007). It can also reduce the amount of other bile acids that can be harmful to liver cells when levels are elevated. (Wolkoff AW and Cohen, 2003.).

Data on the interaction between cholesterol and chenodeoxycholic acid, cited above, have been related to medical and pharmacological problems and did not refer to molecular aspects of the interaction. The latter problems can be solved using the method of nitroxide spin labels (Berliner 1976, 1998, Likhtenshtein 1976, 1993, 2000,'Likhtenshtein et al. 2008, Kocherginsky and Swartz, 1995), which has been proved to solve number problems in chemistry, physics and biology on molecular level. Any motion of a nitroxide radical spin probe (NRSP) is greatly influenced by the molecular dynamics of surrounding molecules and molecular volume of a compound attached to the radical**.** Different NRSP's in the same solution display a functional correlation between a characteristic time of rotation and the molecular volume. These data provide a way to investigate formation of a complex between nitroxide radical spin probe and compound under interest following the labeled complex rotation by ESR technique.

Widely employed parameters which characterize molecular motion of nitroxide is the rotational diffusion correlation time (τ_c), the time of the a molecule rotation for one radian. Modern ESR techniques allows ones to access dynamic processes that are characterized by a wide range of correlation time to measure the molecular motion of nitroxide with a wide range of correlation time, $\tau_c = 10^{2}–10^{-10}$ s and amplitude ending low amplitude high frequency vibration. Fig. 1 shows effect of a nitroxide rotation on its the first harmonic ESR spectra *(V_1),* theoretically calculated in the frame of 3mm X-band ESR spectroscopy. Analysis of experimental spectra allows to calculate the correlation time value.

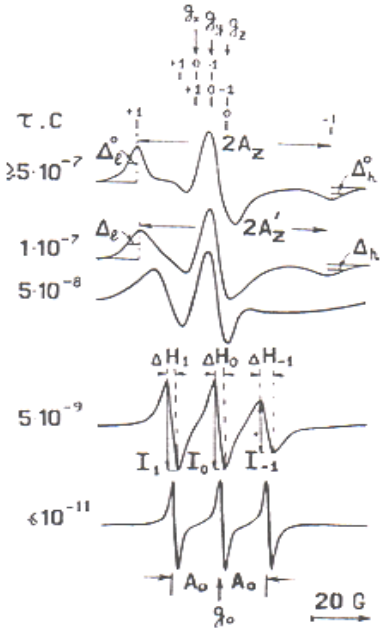


Fig. 1. Theoretically calculated the first harmonic ESR spectra in the 3-cm band (V1) at different values of the nitroxide rotation correlation time. (Likhtenshtein 1993 and references therein).

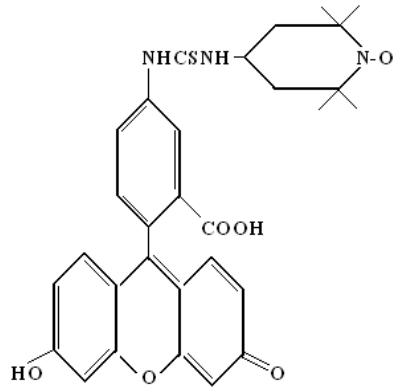
The detail dynamic theory of the nitroxide ESR spectra for nitroxide motion in the fast (τ_c = 10^{-7}-10^{-10} s) and slow regions (τ_c = 10^{-7}-10^{-8} s) was developed by D. Kivelson (Kivelson, 1960) and J. H. Freed (Freed, 1976) and references therein] using the stochastic Liouville equation.

In present work the method of nitroxide spin labels was used for investigation of quantitative interaction of cholesterol and chenodeoxycholic acid in ethanol possessing dielectric properties similar to ones of biological membranes. The basic idea underlying our approach is a labeling of cholesterol solved in ethanol with a spin probes followed by detection of obtained complex by Electron Spin Resonance (ESR) technique. Addition to the solution of chenodeoxycholic acid resulted in superseding of the probe from the complex that is easily monitored by the free probe ESR spectra.

Materials and methods.

Cholesterol and chenodeoxycholic acid were purchased from Sigma-Aldrich was a gift of Drs. V.V. Martin and A.L. Weis (Lipitek International, Inc. USA).

Spin probe I



R

Experiments

In a typical experiment, one ml solution of ethanol containing of cholesterol (3.6×10^{-2} M) and spin probe I ($4\cdot10^{-3}$ M) was incubated for 48 hours in ambient temperature. After addition of chenodeoxycholic acid in amount of (3×10^{-4}), (2.2×10^{-3} M) and (3.3×10^{-3} M), the continuous wave spectra of electron spin resonance (CW ESR) in X-band range were taken in the following conditions: the magnetic field scanning 40 G/min, the high frequency (HF) modulation amplitude 0.3 G, the HF modulation frequency 100 kHz and the time constant 0.1 s.

Analysis of ESR data

For the estimation apparent rotation correlation time the following equations can be used in the region of the fast motion (Kivelson, 1960)

$$\tau_c = \frac{\left[\sqrt{\frac{J_0}{J_{-1}}} - 1\right]\Delta H_0}{3.6 \times 10^9}\ s \quad (1)$$

where J_0, J_{-1} are the heights of the ESR spectrum hyperfine components, respectively (Fig. 1), and ΔH_0 is the line width of the middle hyperfine component. In the region of slow motion the following equition for the radical isotropic rotation can be used (Freed, 1976):

$$\tau_c = 5.4 \times 10^{-10}[1 - \frac{A_{zz}}{A^0_{zz}}]^{-1.36}\ s \quad (2)$$

where A^0_{zz} and A_{zz} are the z-components of A-tensor for immobilized (determined from the rigid limit spectrum) and mobile nitroxide, respectively.

Results

ESR spectra of solution of the spin label R in the presence of cholesterol is shown in Fig 2(1). The spectra is composed with the centered narrow triplet (component I) and the wide component in the high magnetic field (component II), which are related to radicals rotated in the fast and slow motion regions, respectively (Fig. 1). Estimation correlation time component II using Eq. 2 gave values τ_c = 1.2 10^{-7} s. This component obviously belong to the [Cholesterol - Spin label R] complex. Figs 2(3,3,4) demonstrates that effect of addition of chenodeoxycholic acid on the [Cholesterol -Spin label R] complex in solution 2 resulted in disappearance of component II accompanying appearance the triplet from free probe R with correlation time τ_c = 2.1 10^{-10} s.(calculation by

Eq.1). Thus, in this system, the equilibrium is shifted to the products and stable complex [Cholesterol - chenodeoxycholic acid] is formed.

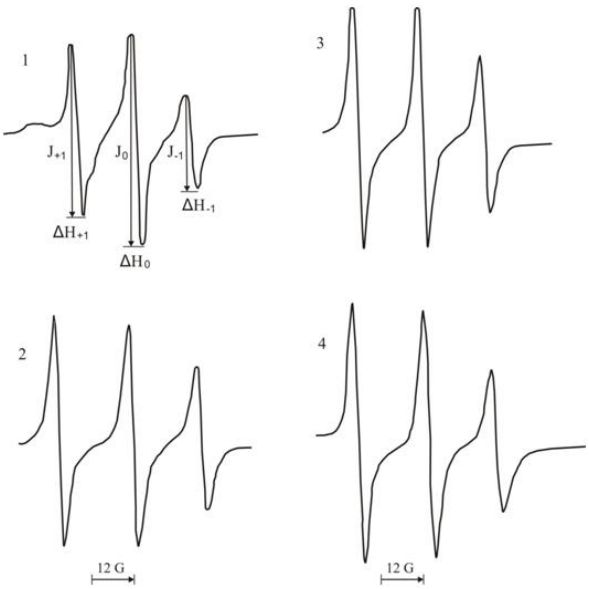


Fig. 2. Continuous wave spectra of electron spin resonance (CW ESR) in X-band range ESR spectra in one ml ethanol solution of cholesterol ($3.6x10^{-2}$ M) and spin probe I ($4x10^{-3}$ M) (1) and after addition of chenodeoxycholic acid in amount of ($3x10^{-4}$) (2), ($2.2x10^{-3}$ M) (3) and ($3.3x10^{-3}$ M) (4).

Discussion

Process occurred in solutions containing cholesterol and spin label R is described by following scheme:

Cholesterol + Spin label ↔ [Cholesterol -Spin label R] (Solution 1)

Spin label R was replaced from the complex [Cholesterol -Spin label I] for chenodeoxycholic acid:
[Cholesterol -Spin label R] + chenodeoxycholic acid ↔ [Cholesterol -chenodeoxycholic acid] + Spin label R (Solution 2)

As shown in results, the value of rotation correlation time for the free label was found to be $\tau_c = 2.1\ 10^{-10}$ s, while for the complex [Cholesterol -Spin label R] this value is markedly high ($\tau_c = 1.2\ 10^{-7}$ s). According to the Stoks-Einstein low, in solution the correlation time of a rotation particle rotating isotropically is proportional to its volume. Therefore, in the ethanol solution the cholesterol are packed in domains of about hundred molecules each

The cholesterol ethanol solution can be considered as a convenient model for study interaction of chenodeoxycholic acid with cholesterol in cholesterol-rich vesicles, and lipid protein complexes. For example, an analysis of the internal structure and dynamics of a polyunsaturated lipid bilayer composed of 1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine containing 29 mol % cholesterol carried out by neutron diffraction, 2H-NMR and 13C-MAS NMR showed that cholesterol molecules are arranges in the membranes with the A-ring near the lipid glycerol and the terminal methyl groups 3 A° away from the bilayer center, that is in the superficial portions. By means of a whole set spin probes, with the nitroxide doxyl fragment on various position, it was shown that the superficial portions of lipid membranes were characterized by a polarity that correspond approximately to that of ethanol solution the cholesterol are packed in domains of about hundred molecules each. (Griffith and Jost, 1976).

There are evidences for existence of cholesterol domains in model and biological membranes. As an example cholesterol is not randomly distributed in either model or biologic membranes. (Schroeder et al., 1991). Membrane cholesterol appears to be organized into structural and kinetic domains or pools. Cholesterol-rich and poor domains can even be observed histochemically and physically isolated from epithelial cell surface membranes. A model describing the mechanisms of cholesterol efflux from cell plasma membrane to high density lipoprotein (HDL) particles suggesting the suggesting the existence of heterogenous domains of cholesterol within plasma membranes has been reported (Rothblat et.al., 1992). It was proposed that cholesterol efflux from cell membranes is influenced by three factors: 1) the distribution of cholesterol between cholesterol-rich and cholesterol-poor membrane domains, 2) the diffusion of cholesterol molecules through the extracellular unstirred water layer, and 3) the transient interaction of segments of the amphipathic helix of the

HDL apolipoprotein with cholesterol-poor membrane domains resulting in enhanced cholesterol efflux.

Conclusion

The nitroxide spin probe method demonstrated that the cholesterol in ethanol solution forms stable complex with chenodeoxycholic acid. Revealing the cholesterol domains in the ethanol solution opened the way for quantitative investigation such aggregates and its interaction with chenodeoxycholic acid and other molecules in model and biological membranes using the version of spin label method which has been developed in this work. The method also can be useful for study of interaction of chenodeoxycholic acid with cholesterol in cholesterol-rich vesicles and lipid protein complexes and for kinetics of formation and dissolving of cholesterol gallstones

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