CROATICA CHEMICA ACTA CCACAA **71** (1) 189–198 (1998)

ISSN-0011-1643
CCA-2488

Original Scientific Paper

The Influence of Lower Alcohols on the Surface Lipid Monolayer in LDL

Marina Kveder,[a] *Greta Pifat,*[a] *Slavko Pečar,*[b,c] *and Milan Schara*[b]

[a]*Ruđer Bošković Institute, P.O.B. 1016, 10001 Zagreb, Croatia*
(e-mail: kveder@rudjer.irb.hr)

[b]*J. Stefan Institute, 61000 Ljubljana, Slovenia*

[c]*Faculty of Pharmacy, University of Ljubljana, 61000 Ljubljana, Slovenia*

Received July 2, 1997; revised October 3, 1997; accepted October 27, 1997

Interaction of methanol, ethanol, propanol and butanol with human plasma low density lipoproteins (LDL) was studied in this work. The surface lipid monolayer of LDL was spin labeled and the electron spin resonance (ESR) spectra were measured in the presence and absence of alcohols. The decomposition of the complex ESR spectra was performed *via* theoretical simulations of experimental data. The results gained from this study indicate that the influence of alcohol could be observed through the changes of lipid ordering in the surface of LDL monolayer. This observation supports the hypothesis on the mechanism of alcohol action through its interference with lipid-protein interactions at the level of macromolecular surface.

INTRODUCTION

Various species circulating in the bloodstream can undergo mutual interactions. This is especially important when the influence of alcohol on the lipid-protein assemblies is considered. So far, there is still a disagreement as to whether the primary site to be affected by alcohol is the lipid or protein phase of the biosystem.[1]

Reported data[2] claim that chronic abusers can survive bloodstream ethanol levels in excess of 0.3 M. On the other hand, it is known[3] that the level of low density lipoproteins (LDL) in human serum is about 1.2 μM. Considering this LDL concentration range it could be expected that, besides

other molecules circulating in the blood, also the interaction of the bloodstream alcohol with LDL particles is possible. Therefore, it is important to understand the molecular mechanism of their interaction. In our previous work,[4] the interaction of lower alcohols with human plasma LDL was addressed. Using the electron spin resonance (ESR) spectroscopic method, the experimental evidence of alcohol-LDL interaction was offered. The LDL particles were spin labeled at the level of apoB and the ESR data were explained following the arguments of the phospholipid mediated mechanism of alcohol action, which results in the protein conformational change. In order to continue along the same line of investigation, in this work the surface lipid monolayer of LDL was spin labeled and the ESR spectra analyzed in the presence of different alcohols. The aim of the study was to detect whether the changes in the ordering of the surface lipid phase in LDL are induced upon the interaction with alcohol.

MATERIALS AND METHODS

Materials

The spin label 1-oxyl-2,2,-dimethyl-4-undecyl-4-methoxy-carbonylpropyl -oxazolidine (MeFASL(10,3)) was synthesized by one of the authors (S.P.) according to the published procedure.[5]

Alcohols were of *p.a.* purity and purchased from Kemika, Zagreb. In order to get the desired final alcohol concentration in the lipoprotein solution, a double alcohol concentration of the stock solution was prepared and then mixed with the sample in 1:1 ratio.

All the experiments were performed in the 0.1 M Tris-HCl buffer (1 g/L EDTA; pH = 7.4).

Isolation of LDL

Human plasma was obtained from two female normolipidemic donors and lipoproteins were isolated according to the published procedures.[6,7] To avoid oxidation of lipoproteins, EDTA was present in all preparation steps and all buffers were flushed with nitrogen.

Purity of the lipoprotein samples was checked by electrophoresis.

The LDL concentration was determined gravimetrically by dry weight estimation including correction for the salt content of the buffer.

Spin Labeling of Lipoproteins

The spin label dissolved in ethanol was deposited as a dry thin film on the wall of the glass tubing into which the LDL sample was added. The

maximal expected molar ratio of nitroxide dissolved in LDL versus the estimated surface lipid content was always kept less than 1:100. Incubation under moderate shaking lasted for 5 h at 30 °C. After exhaustive dialysis in Tris-HCl buffer, the LDL samples were filtered through 450 nm sterile filters and the ESR measurements were started.

ESR Spectroscopy and Analysis

Experiments were performed in glass capillaries (1 mm inner diameter) on an X-band Varian E-109 ESR spectrometer. The following settings were used: microwave power, 10 mW; modulation amplitude, 0.1 mT; modulation frequency, 100 kHz and scan range 20 mT. The acquisition and the analysis of the ESR data were performed using the EW Scientific Software Services program.[8] All ESR spectra were measured at t = 37 °C.

For a detailed analysis of the experimental data, computer simulation of the ESR spectra was performed assuming a fast nitroxide rotation about the normal on the lipoprotein surface.[9] This framework allows the use of the order parameter, S, defined as:

$$S = \frac{\kappa\left(\left\langle \boldsymbol{A}_{\parallel}\right\rangle - \left\langle \boldsymbol{A}_{\perp}\right\rangle\right)}{\left(\boldsymbol{A}_{\parallel} - \boldsymbol{A}_{\perp}\right)}$$

where $\boldsymbol{A}_{\parallel}$ and $\boldsymbol{A}_{\perp}$ are the crystal values of the components of hyperfine tensor while the values in brackets are obtained from the best fit. The parameter κ takes into account the polarity correction for the $\boldsymbol{A}$ hyperfine tensor.

RESULTS

The molecule of MeFASL(10,3) and the assumed model of its incorporation into the surface lipid monolayer of LDL is presented in Figure 1. This spin label probes the phospholipid acyl chain region of LDL and the orientation of the nitrogen 2pπ orbital in the paramagnetic group of nitroxide is preferentially parallel to the long molecular axis which coincides with the normal to the lipoprotein surface. The ESR spectrum of the spin labeled native LDL is shown in Figure 2 along with simulation of the experimental data. This approach revealed the superimposition of two components in the ESR spectrum, denoted as A and B, with different order parameters, *i.e.* S_A = 0.15 and S_B = 0.5 and the corresponding weight factors of W_A = 0.55 and W_B = 0.45 respectively (insert to Figure 2). In the presence of 0.3 M methanol, ethanol and propanol, no substantial changes of the spin labeled LDL spectra could be observed (Figure 3) so that the same fitting parameters as

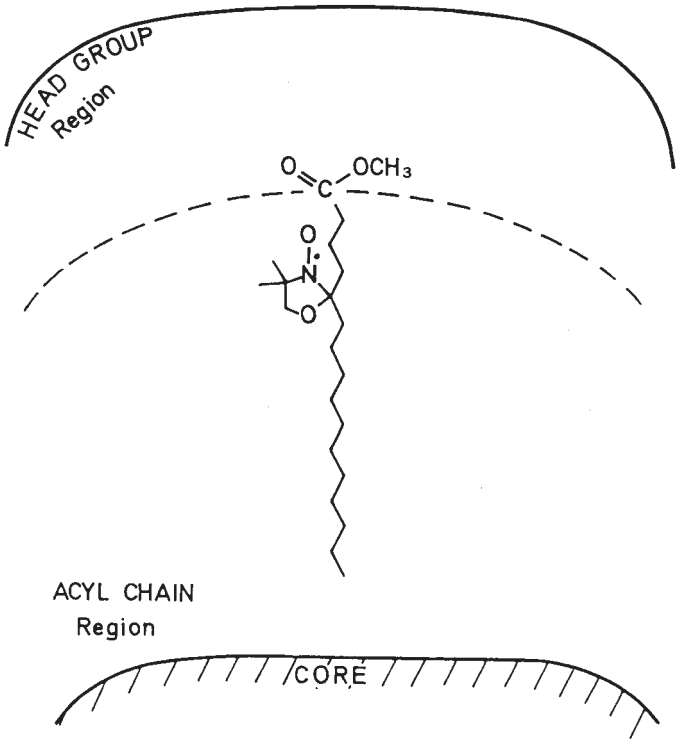

Figure 1. The molecule of MeFASL(10,3) and the assumed model for its incorporation into the surface lipid monolayer of LDL.

in the case of the native samples are applicable (Table I). However, in the experiments with 0.3 M butanol (Figure 3), the ESR spectrum indicates an increase in disorder which is reflected in an increase of the weight factor for the component with the smaller order parameter (Table I). This phenomenon can be observed for methanol and ethanol if the concentration of alcohol is increased by one order of magnitude (Table I). In the presence of 3 M propanol, the ESR spectrum shows denaturation of the LDL particle (Figure 4), where both components indicate an extremely low level of ordering.

In order to check whether the empirical rotational correlation time, τ, deduced from the ESR spectra[10] for MeFASL(10,3) incorporated in LDL is sensitive to the presence of alcohol, the following equation was used:

$$\tau = K\,\Delta H(0)\,[(I_0/I_{-1})^{1/2} - 1] \qquad (1)$$

where K is the proportionality constant, I_0 and I_{-1} are the first derivative amplitudes of the central and the high field hyperfine components in the

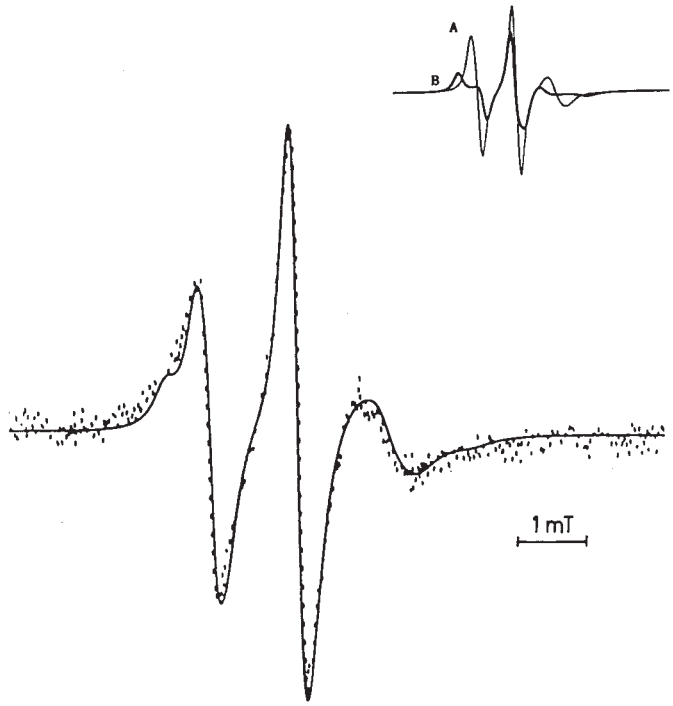

Figure 2. The ESR spectrum of native LDL spin labeled with MeFASL(10,3). The concentration of LDL was 12.8 μM. The experimental data (dotted) were fitted (line) with two superimposed components A and B (Insert to Figure 2) which contribute to the total spectrum with weight factors $W_A = 0.55$ and $W_B = 0.45$ respectively. The following parameters were used for the components: order parameter, $S_A = 0.15$, $S_B = 0.5$; rotational correlation time, $\tau_A = \tau_B = 1.5$ ns; half width at half maximum of the Lorentzian line shape for $m = 0$, $\Gamma_A = \Gamma_B = 0.2$ mT; polarity correction in the $\boldsymbol{A}$ hyperfine tensor, $\varkappa_A = 1.07$, $\varkappa_B = 1.05$.

ESR spectrum, respectively, and $\Delta H(0)$ is the corresponding linewidth. These results are presented in Table II. Calculated τ values suggest that the rotational correlation time extracted directly from the spectral amplitudes and linewidth can reliably discriminate only between the native and highly denatured particles.

DISCUSSION

In this work, the influence of alcohols, with short alkyl chains, on LDL particles was studied at the level of the lipoprotein surface monolayer (Figure 1). Methanol, ethanol, propanol and butanol were applied at two concentrations. *i.e.* 0.3 M, which represents the physiological upper limit, and 3 M,

TABLE I

Theoretical simulation of the ESR spectra of spin labeled LDL exposed to different alcohol concentrations

[a]log(part.coeff.)	Type of alcohol							
	–	Methanol –0.73		Ethanol –0.32		Propanol 0.34		Butanol 0.88
Concentration, mol dm^3	0	0.3	3	0.3	3	0.3	3	0.3
W_A	0.55	0.55	0.65	0.55	0.65	0.55	0.65	0.65
W_B	0.45	0.45	0.35	0.45	0.35	0.45	0.35	0.35
S_A	0.15	0.15	0.15	0.15	0.15	0.15	0.01	0.15
S_B	0.5	0.5	0.5	0.5	0.5	0.5	0.05	0.5
τ_A, ns	1.5	1.5	1.5	1.5	1.5	1.5	0.3	1.5
τ_B, ns	1.5	1.5	1.5	1.5	1.5	1.5	0.5	1.5
κ_A	1.07	1.07	1.07	1.07	1.07	1.07	1.0	1.07
κ_B	1.05	1.05	1.05	1.05	1.05	1.05	1.07	1.05
[b]Γ_A, mT	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2
Γ_B, mT	0.2	0.2	0.2	0.2	0.2	0.2	0.15	0.2

[a]the experimentally determined partition coefficients for each alcohol in the octanol/water system quoted by W. D. Stein and W. R. Lieb, *Transport and Diffusion across Cell Membranes*, Academic Press, 1986, p. 88.

[b]Γ denotes the half width at half maximum of the Lorentzian line shape for $m = 0$.

TABLE II

The empirical rotational correlation time, τ, for MeFASL(10,3) incorporated in LDL in the presence of alcohol deduced from the ESR spectra

Type of alcohol	Concentration of alcohol	τ, ns*
–	0	2.9
methanol	0.3 M	3.0
ethanol	0.3 M	2.8
propanol	0.3 M	2.9
butanol	0.3 M	2.5
methanol	3 M	3.0
ethanol	3 M	2.7
propanol	3 M	0.7

*The reproducibility of τ determination from equation (1) is within the precision of determination $\Delta H(0)$, I_o, I_{-1} *i.e.* ± 0.2 ns.

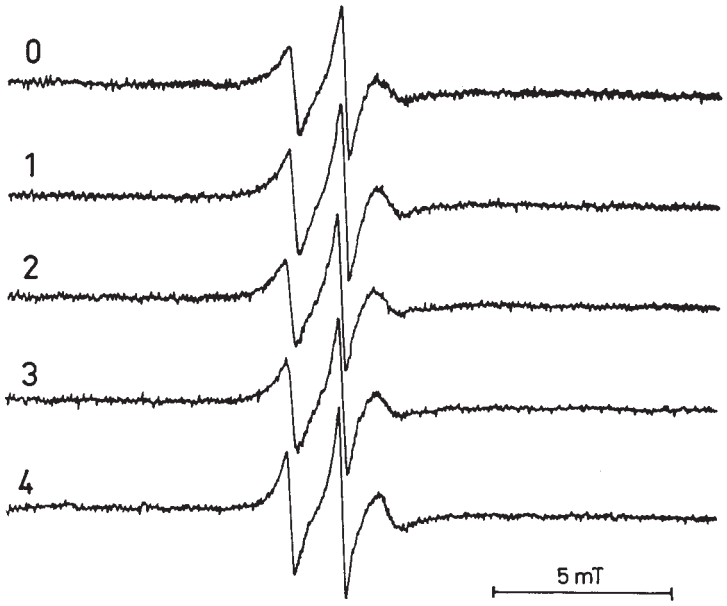

Figure 3. Influence of different lower alcohols on the ESR spectra of spin labeled LDL. In all experiments, the concentration of LDL was 12.8 μM and the concentration of alcohol was 0.3 M. The numbering on each spectrum indicates the presence of a certain alcohol in the experiment according to: »0« (control sample), »1« (methanol), »2« (ethanol), »3« (propanol) and »4« (butanol).

which provides the extreme denaturing condition. The interaction of alcohols with LDL was followed by analyzing the induced changes in the ESR spectra *via* theoretical simulation of the experimental data.

In the native LDL samples (Figure 2), at least two domains of different ordering could be assigned to populate the monolayer, which is in accordance with previous findings.[9] The order parameter, S = 0.5, indicates a rather ordered domain, whereas S = 0.15 reveals the domain with a less motional restriction which slightly predominates (weight factor 0.55). The existence of domains could be understood in terms of the complex chemical composition of constituent molecules in the particle.[11] Namely, the length and degree of saturation of fatty acid building blocks in the monolayer govern the packing properties of phospholipids with cholesterol.[12] In the presence of 0.3 M methanol, ethanol and propanol (Figure 3), the ESR spectra do not indicate any visible changes of the lipid state. However, in the presence of 0.3 M butanol (Table I), the increase of the weight factor to 0.65 for the component with the smaller order parameter could be observed. Thus, the lipid monolayer became less ordered in the presence of butanol. Since different alcohols were used at the very same molar concentrations, the difference in

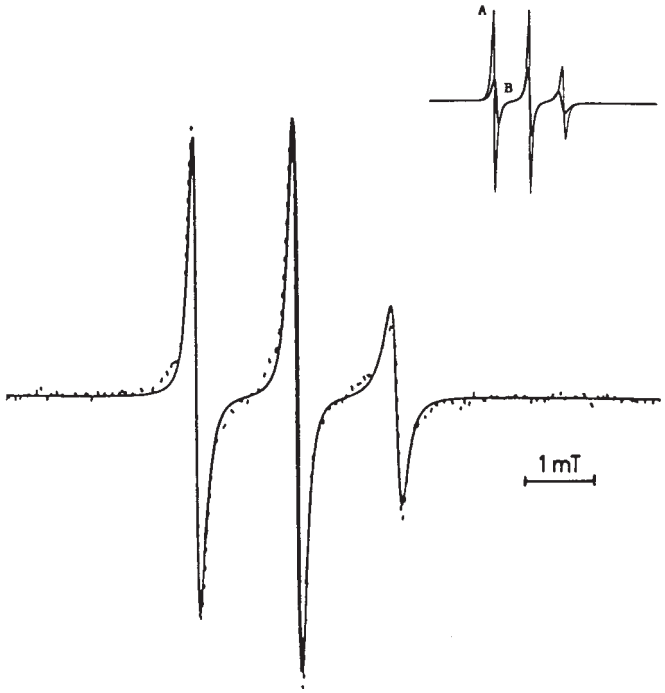

Figure 4. The ESR spectra in the presence of 3 M propanol.
The concentration of LDL was 12.8 μM. The experimental data (dotted) were fitted (line) with two superimposed components A and B (Insert to Figure 4) which contribute to the total spectrum with weight factors 0.65 and 0.35, respectively. The following parameters were used for the components: order parameter, $S_A = 0.01$, $S_B = 0.05$; rotational correlation time, $\tau_A = 0.3$ ns, $\tau_B = 0.5$ ns; half width at half maximum of the Lorentzian line shape for $m = 0$, $\Gamma_A = 0.1$ mT, $\Gamma_B = 0.15$ mT; polarity correction in the $\boldsymbol{A}$ hyperfine tensor, $\varkappa_A = 1.0$, $\varkappa_B = 1.07$.

the length of the respective alkyl chains is responsible for the induced perturbation in the LDL monolayer. This observation is consistent with the results of similar experiments at the apoB level.[4] Therefore, the explanation for these findings is searched in the involvement of alcohols in the lipid-protein interactions. Namely, the incorporation of alcohol at the lipid-water interface displaces the water molecules bound at the macromolecular surface.[13] This event provokes a modulation of the lipid packing free volume and induces a change in the protein conformation. Thus, the difference in the lipophilicity of alcohols, which governs their solubility in the monolayer of the LDL particle, is the principal determinant of the alcohol-LDL interactions. To induce the change in the lipid chain order, the concentration of methanol and ethanol should be increased by one order of magnitude (Table I). Furthermore, in the presence of 3 M propanol, the ESR spectrum reflects

LDL particle denaturation (Figure 4). Both spectral components indicate the presence of extremely disordered domains (S_A = 0.01 with weight factor W_A = 0.65 and S_B = 0.05 with weight factor W_B = 0.35). Hence, these observations show that the influence of alcohol could be observed through the changes of lipid ordering in the surface of the LDL monolayer.

It should be stressed that the conclusions gained from this study could be derived only through theoretical simulations of the experimental data, allowing decomposition of the complex spectra. For comparison, the empirical rotational correlation time extracted directly from the amplitudes and linewidths in the ESR spectra (Table II) should be taken only as a rough estimation of the order of magnitude for the time scale describing molecular motions.

In conclusion, together with the results of our previous study,[4] the ESR method provided the experimental evidence of LDL interaction with alcohols. Furthermore, the explanation of the molecular mechanism of this process is proposed as the disruption of the lipid-protein interaction when alcohol competes with water molecules for hydrogen bonding at the LDL surface. Consequently, changes in the lipid packing are induced and propagated in the perturbation of the native protein conformation of apoB.

Acknowledgments. – This work was partly supported by the Ministry of Science of the Republic of Croatia, by the Ministry of Science and Technology of the Republic of Slovenia and partly by the joint Slovenia-Croatia project: »Structure and dynamics of synthetic and biological species«.

REFERENCES

1. R. W. Peoples, C. Y. Li, and F. F. Weght, *Annual Review of Pharmacology and Toxicology* **36** (1996)185–201.
2. I. Diamond, *Cecil Textbook of Medicine*, W. B. Saunders (Ed.), Philadelphia, 1992, pp. 44–47.
3. H. Esterbauer, J. Gebicki, H. Puhl, and G. Jürgens, *Free Rad. Biol. Med.* **13** (1992) 341–390.
4. M. Kveder, G. Pifat, S. Pečar, and M. Schara, *Chem. Phys. Lipids* **87** (1997) 125–135.
5. W. L. Hubbell and H. M. McConnell, *J. Am. Chem. Soc.* **93** (1971) 314–326.
6. G. Jürgens, H. F. Hoff, G. M. Chisolm and H. Esterbauer, *Chem. Phys. Lipids* **45** (1987) 315–336.
7. H. Puhl, G. Waeg, and H. Esterbauer, *Methods in Enzymology*, L. Packer (Ed.), Academic Press, 1994, pp. 425–441.
8. P. D. Morse II, *Biophys. J.* **51** (1987) 440a.
9. M. Kveder, G. Pifat, S. Pečar, and M. Schara, *Chem. Phys. Lipids* **70** (1994) 101– 108.
10. D. Kivelson, *J. Chem. Phys.* **33** (1960) 1094–1106.
11. M. Kveder, G. Pifat, S. Pečar, and M. Schara, *Chem. Phys. Lipids,* **85** (1997) 1–12.
12. J. F. Tocanne, L. Cezanne, A. Lopez, B. Piknowa, V. Chram, J. F. Tournier, and M. Welby, *Chem. Phys. Lipids* **73** (1994) 139–158.
13. J. A. Barry and K. Gawrisch, *Biochemistry* **33** (1994) 8082–8088.

SAŽETAK

Utjecaj nižih alkohola na površinski lipidni monosloj u LDL

Marina Kveder, Greta Pifat, Slavko Pečar i Milan Schara

Proučavano je međudjelovanje metanola, etanola, propanola i butanola s lipoproteinima niske gustoće (LDL) izoliranima iz ljudske plazme. Površinski monosloj LDL spinski je označen, a spektri elektronske spinske rezonancije (ESR) mjereni su u prisutnosti i odsutnosti alkohola. Rastav složenih spektara ESR proveden je teorijskom simulacijom eksperimentalnih podataka. Rezultati dobiveni u ovom istraživanju pokazuju da se utjecaj alkohola može opaziti kroz promjenu uređenosti lipida površinskog monosloja LDL. To opažanje podržava hipotezu o mehanizmu djelovanja alkohola kroz njegovu interferenciju s međudjelovanjem lipid/protein na makromolekulskoj površini.