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The ESR Kinetic Study of Lipid Phase in HDL

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Two main high-density lipoprotein subfractions, HDL_2 and HDL_3, were spin labelled with TEMPO which partitions both in aqueous and lipid phase. The dynamics of the lipid phase was monitored *via* the reduction of incorporated TEMPO with ascorbic acid. The reduction of the paramagnetic nitroxide into the nonparamagnetic hydroxilamine form decreases the ESR signal with time. The reduction curves show complex behaviour while the partition coefficients of TEMPO remain unchanged during the reaction. The reduction process in the samples containing HDL particles proceeds faster than in the aqueous solution of pure reactants, *e.g.* spin label and ascorbic acid. In order to explain experimental data the model for reduction of TEMPO by ascorbic acid is proposed. It assumes that the processes taking place in these heterogeneous systems are determined by the overall reaction rate, which depends on the local concentrations of the reactants, as well as on their transport properties in the particular phases.

Key words: ESR, lipoproteins, HDL, reduction kinetics.

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INTRODUCTION

Ever since the close interrelation between atherogenesis and disorders of lipid metabolism has been proven, the molecular structure and function interdependence in lipoprotein particles has been of great interest.[1,2]

The spectroscopic studies led to the generally accepted model for all lipoprotein particles, the so-called »oil drop model«.[3] The core of neutral lipids (triacylglycerols and cholesteryl esters) is encapsulated by an amphipathic monolayer of polar lipids (phospholipids and cholesterol). The thickness of the monolayer is determined by the length of acyl chains of phospholipids and is similar in different lipoprotein particles. As a consequence, in smaller lipoprotein particles the polar phospholipid heads are more apart, leaving space for water molecules to be included into the monolayer. The amphipathic protein, which embraces the particle by the hydrophobic interaction with phospholipid, assures the stability of the structure, but does not penetrate into the monolayer more than half its depth.[4] The smaller the lipoprotein particle, the larger is the protein/lipid weight ratio. None of the lipoprotein fractions in the serum is homogeneous with respect to the unique chemical composition. The reason is in the design of these macromolecular entities that are responsible for the mass transport so that the catabolic process controls the content and the structure of individual particle.

High-density lipoprotein (HDL) is the smallest and the most abundant human plasma lipoprotein. It has a major role in reverse cholesterol transport. HDL is a heterogeneous group of particles[5,6] and the clinical significance differs with the subclasses. Different separation methods define slightly different subclasses according to physicochemical properties. It is common to define two main HDL subfractions HDL_2 and HDL_3. There are similarities in structure of these particles: predominance of monolayer over core volume, high protein/lipid ratio and higher content of polar over neutral lipids[7,8,9] but at the same time there is a great functional diversity especially with respect to the coronary heart disease.[10] It is the concentration of HDL_2 particle in blood that shows the inverse relationship to the incidence of atherosclerosis.

Here we report the study of nitroxide reduction with ascorbic acid in spin labelled human HDL_2 and HDL_3 in order to examine the dynamics of particles surfaces, which could play a role in their physiological function.

EXPERIMENTAL

Lipoprotein Isolation

The human serum lipoproteins in the density range of 1.063–1.125 g/cm^3 (HDL_2) and 1.125–1.21 g/cm^3 (HDL_3) have been isolated by sequential ultracentrifugation of fresh-drawn plasma from four normolipidemic female donors as already described.[11] The purity of the lipoprotein samples was checked by electrophoresis and the concentrations of lipoprotein solutions were determined gravimetrically.

The spin label TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) was synthesized according to the published procedure.[12]

Chromium oxalate was prepared according to Berg and Nesbitt 1979.[13]

Ascorbic acid was purchased from Pliva Chemical Company. For each experiment it was dissolved in the buffer immediately before the measurement.

The lipoprotein samples and all the dilutions in the experiments were performed in 0.1 mol/L Tris-HCl buffer, pH = 7.4 with 1 g/L of EDTA except chromium oxalate. It was prepared in distilled water to match the physiological osmolarity.

Spin Labelling of Lipoproteins

To perform a comparative study of two HDL subfractions, the concentration of both lipoprotein samples was adjusted such that the estimated concentration of the monolayer lipids was 0.02 mol/L. TEMPO dissolved in Tris-HCl buffer was added to the lipoprotein suspension. The molar ratio of TEMPO dissolved in lipoproteins *versus* surface lipid content was kept approximately 1 : 50. The equilibrium distribution of TEMPO was reached within 30 minutes, as independently checked by monitoring the time dependence of ESR signal over a longer period of time.

Analysis of Fatty Acid Composition

In order to define chemical structure characteristics of the HDL particles the analysis of fatty acid composition of phospholipids, triacylglycerols and cholesteryl esters was performed according to the published procedure.[14,15]

Chromium Oxalate Measurements

The paramagnetic agent chromium oxalate was added to the spin labelled lipoprotein solutions in order to prove that TEMPO does partition in HDL particles. The consequence of the interaction between TEMPO and chromium oxalate is the line broadening of the ESR spectrum. Since chromium oxalate does not penetrate into the HDL particle, the remaining component in the spectrum is assigned to the spin label molecules dissolved in the lipid phase of HDL, as confirmed by hyperfine splitting. In the final solution the concentration of chromium oxalate was 60 mmol/L.

Nitroxide Reduction with Ascorbic Acid

The kinetics of the nitroxide reduction with ascorbic acid monitors the dynamics of the lipid phase of two high-density lipoprotein subfractions. For that purpose the concentration of the ascorbic acid *versus* TEMPO was in nearly threefold excess. The process was followed by the decrease in the intensity of ESR spectra that were nor-

malised to the strong pitch standard. The spectrum at zero time was obtained with the lipoprotein sample in which the aliquot of buffer instead of ascorbic acid was added. The intrinsic reduction rate of TEMPO with ascorbic acid was deduced from measurements in the pure mixture of reactants.

ESR Spectroscopy

All spectra were recorded at (37 ± 1) °C with Varian E-109 ESR spectrometer, operating at: frequency 9.62 GHz, microwave power 10 mW, modulation amplitude 0.1 mT and modulation frequency 100 kHz. The acquisition of ESR spectra was performed using EW Scientific Software Service program.[16]

For the data analysis the simulation of experimental spectra was performed with two superimposed Gaussian type spectra. The ESR spectrum, S, was presented as a superimposition of the spectra corresponding to the spin probe residing in the hydrophobic (S_h) and hydrophilic (S_p) environments:

$$S = f_T S_h + (1 - f_T)S_p \quad (1)$$

where f_T, the partition parameter, denotes the contribution of the hydrophobic component in the total ESR spectrum.[17]

RESULTS

Chemical Composition of Lipids

The result of chemical composition analysis of fatty acids (FA) in HDL_2 and HDL_3 particles is presented in Table I. In the first part (Table Ia) the abundance of certain FA is expressed as its mole fraction towards the total amount of all FA. In the second part (Table Ib) the data are presented as the mole fraction of the specific FA in the core or in the monolayer with respect to the total amount of that particular FA per lipoprotein particle. The calculation was performed according to the oil-drop model. Namely, the FA of triacylglycerol and cholesteryl ester are presented as a content of the core, while the FA of phospholipids are attributed to the monolayer. In the analysis of FA content the distribution of unsaturated FA between the monolayer and the core is extremely important due to their ability to disturb the lipid packing in the observed phase.[18] Our analysis demonstrates (Table Ia) a balance in the content of saturated *versus* unsaturated FA *pro* HDL_2 particle. Regarding their distribution between the monolayer and the core it is evident (Table Ib) that the majority of the unsaturated FA constitutes the core lipids while leaving saturated FA predominantly in the monolayer.

In HDL_3 particle 66% are unsaturated FA, almost equally distributed between the surface monolayer and the core (Table I). Therefore, in comparison with HDL_2 in HDL_3 particle one would expect a less ordered, more fluid monolayer.[19] It should be also stressed that the substantial part of the mono-

TABLE I

Analysis of fatty acid contents in HDL_2 and HDL_3 particles

a) Composition of FA (mole fractions/%) per lipoprotein particle

Lipoprotein	Fatty acid									
	14 : 0	16 : 0	18 : 0	16 : 1	18 : 1	18 : 2	18 : 3	20 : 4	22 : 6	Total
HDL_2	3.66	32.94	13.60	4.22	20.83	20.75		3.98		99.98
HDL_3	0.98	27.10	11.58	4.20	22.84	30.24	0.20	2.58	0.20	99.92

b) Relative distribution of FA (mole fractions/%) between the monolayer and the core per lipoprotein particle

	Fatty acid								
	14 : 0	16 : 0	18 : 0	16 : 1	18 : 1	18 : 2	18 : 3	20 : 4	22 : 6
HDL_2									
Monolayer		72.85	93.20		27.03			9.02	
Core	100	27.15	6.8	100	72.96	100		90.99	
Total	100	100	100	100	100	100		100	
HDL_3									
Monolayer	69.75	72.95	91.61	25.72	48.03	43.27		84.77	100
Core	30.25	29.05	8.4	74.27	51.97	57.73	100	15.23	
Total	100	100	100	100	100	100	100	100	100

layer volume is occupied by the apoproteins. It is generally accepted[20] that HDL apoproteins intrude into the lipid phase with 2/3 of their volumes so that protein/lipid hydrophobic interactions are to be considered when discussing the kinetics taking place at the surface of these lipoproteins.[21]

Spin Label Partitioning in HDL

The ESR spectra of TEMPO dissolved in lipoprotein solutions are presented on Figure 1. The complex experimental spectra have been resolved *via* theoretical simulation described previously.[17] The component with larger hyperfine splitting is ascribed to TEMPO dissolved in the polar environment, while the one with smaller hyperfine splitting is attributed to the probe molecules dissolved in lipid part of lipoprotein particle. The spectrum of that component was achieved in the experiment with fast relaxing paramagnetic agent (Figure 1, Insert). The theoretical simulations of the equilibrium spectra resulted in the partition parameters defined in (1): $f_T = 0.20$ and $f_T = 0.28$ for HDL_2 and HDL_3, respectively.

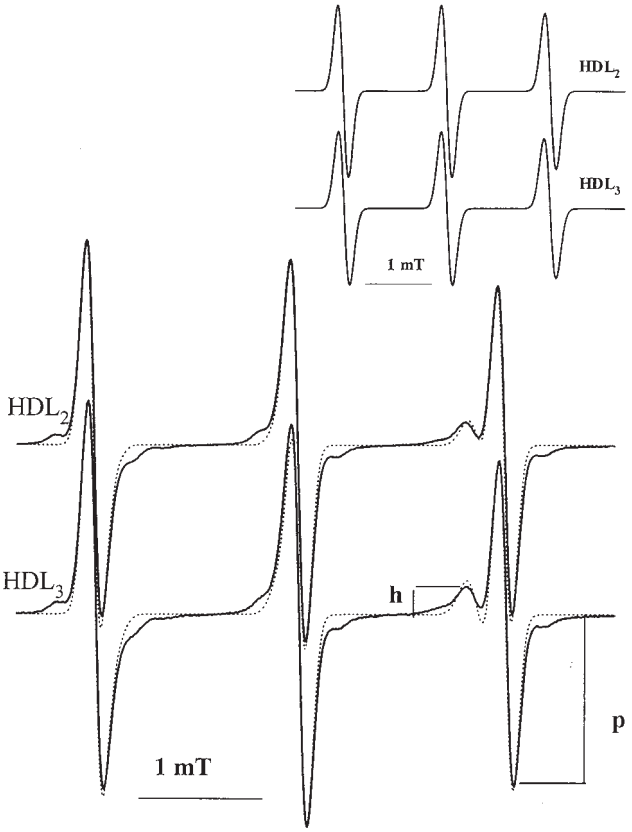

Figure 1. The equilibrium spectra of TEMPO partitioning in HDL_2 and HDL_3. The experimental spectra are presented with a full line whereas the theoretical simulations are depicted with the dotted line. The following concentrations were used in the experiments: c(TEMPO) = 0.41 mmol/L; $c(HDL_{2)}$ = 0.127 mmol/L; $c(HDL_3)$ = 0.334 mmol/L. The fitting parameters are:
a) polar component for both lipoproteins: τ_c = 0.01 ns, linewidth of the central peak = 0.13 mT, hyperfine splitting = 1.72 mT and g factor = 2.0023;
b) hydrophobic part for HDL_2: τ_c = 0.03 ns, linewidth = 0.17 mT, hyperfine splitting = 1.58 mT, g factor = 2.0027 and f_T = 0.20; for HDL_3: τ_c = 0.03 ns, linewidth = 0.18 mT, hyperfine splitting = 1.57 mT, g factor = 2.0028 and f_T = 0.28.
The insert: the spectra of TEMPO dissolved in the lipid phase of HDL particles in the presence of chromium oxalate in the lipoprotein solutions.

Reduction of TEMPO by Ascorbic Acid

The ascorbic acid was used to monitor the dynamics of TEMPO equilibration in lipoproteins exposed to the nonequilibrium conditions. The paramagnetic nitroxide is reduced to the nonparamagnetic hydroxylamine form

in the reaction with ascorbic acid and therefore the ESR signal of TEMPO decreases with time (Figure 2). The time course of TEMPO reduction with ascorbic acid is presented on Figure 3. It is interesting to note that the reaction proceeds faster in the samples containing HDL particles as compared to the pure mixture of reactants (spin label and ascorbic acid). At the same time the partition parameter of TEMPO stays unchanged during the reduction in both lipoprotein solutions.

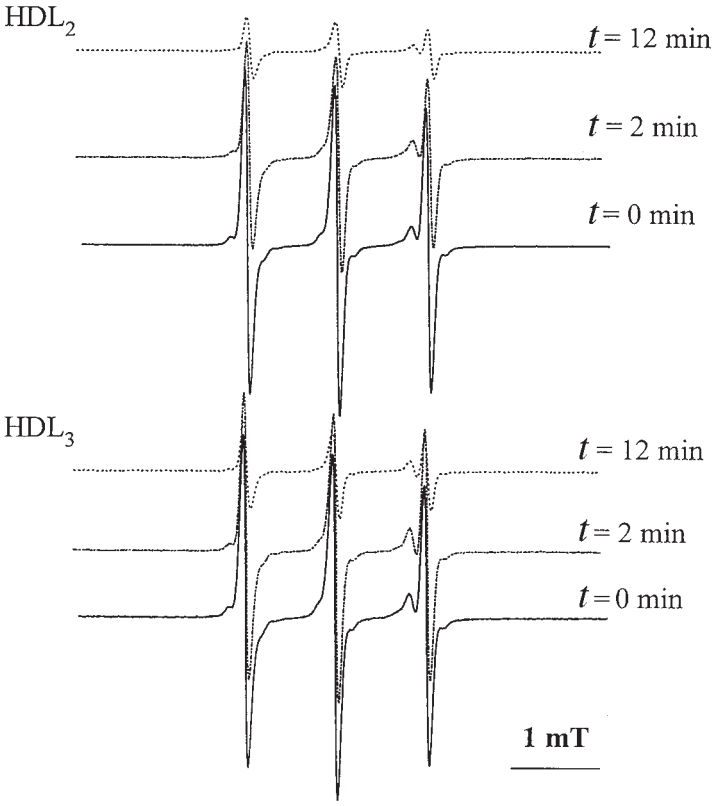

Figure 2. The ESR spectra of nitroxide reduction with ascorbic acid in spin labelled HDL_2 and HDL_3 solutions. The full line represents the control spectra without ascorbic acid. Dashed (dotted) lines denote spectra 2 (12) minutes after the addition of ascorbate, respectively.

Model for TEMPO Reduction in HDL Solution

The complex reduction kinetics of TEMPO in HDL solutions is simulated by the set of coupled differential equations. Here the simultaneous pro-

cesses taking place in the heterogeneous system should be considered. For the comparison, even in the pure system of TEMPO and ascorbic acid the disappearance of the spin label signal depends on three parallel reactions: $SL^* + A \rightarrow SLH + DHA$, $SLH + O_2 \rightarrow SL^*$ and $A + O_2 \rightarrow DHA$.[22] We denoted spin probe (nitroxide) with SL^* and its reduced form (hydroxylamine) with SLH, while A stays for ascorbate and DHA for dehydroascorbate. The dominant spin probe reduction is modulated by oxidation of hydroxylamine, as well as by the oxidation of ascorbic acid in areobic conditions. The equations which satisfactorily describe the proposed reactions in the homogeneous mixture of reactants are:

$$\begin{aligned}
\frac{\mathrm{d}c_{\mathrm{SL}}}{\mathrm{d}t} &= -k_1 c_{\mathrm{SL}} c_{\mathrm{A}} + k_2 c_{\mathrm{SLH}} c_{\mathrm{O_2}} \\
\frac{\mathrm{d}c_{\mathrm{O_2}}}{\mathrm{d}t} &= -k_2 c_{\mathrm{SLH}} c_{\mathrm{O_2}} - k_3 c_{\mathrm{A}} c_{\mathrm{O_2}} \\
\frac{\mathrm{d}c_{\mathrm{A}}}{\mathrm{d}t} &= -k_1 c_{\mathrm{SL}} c_{\mathrm{O_2}} - k_3 c_{\mathrm{A}} c_{\mathrm{O_2}} \\
c_{\mathrm{SLH}} &= (c_{\mathrm{SL0}} - c_{\mathrm{SL}})
\end{aligned} \tag{2}$$

where c_{SL}, $c_{\mathrm{O_2}}$ and c_{A} are the concentrations of TEMPO, oxygen and ascorbic acid in the aqueous solution, respectively. The starting concentrations were $c_{\mathrm{SL}}(t = 0) = c_{\mathrm{SL0}} = 0.4 \times 10^{-3}$ mol/L, $c_{\mathrm{O_2}}(\mathrm{t} = 0) = 2 \times 10^{-4}$ mol/L and $c_{\mathrm{A}}(\mathrm{t} = 0) = 1.1 \times 10^{-3}$ mol/L. Free parameters in the model, k_1, k_2 and k_3 are determined from the best fit of the experimental data.

To extend the model for reduction of spin probe in the homogeneous solution to the samples containing HDL particles, the coexistence of spin label in two environments with different reduction rates was assumed. The system of diferential equations (3) describes the reactions taking place parallely in the aqueous solution and in the particles. The overall reduction rate should depend on the local concentrations of reactants as well as on their transport parameters for the particular phase. We also assume that, due to the hydrophobicity of the lipoprotein particle interior, there could be an alteration in local concentrations of the reactants within as well as on the surface of the particle. Therefore, the set of equations describing the reduction of nitroxide with ascorbic acid in that heterogeneous system is:

$$\frac{\mathrm{d}c_{\mathrm{SL}}}{\mathrm{d}t} = -k_1 c_{\mathrm{SL}} c_{\mathrm{A}} + k_2 c_{\mathrm{SLH}} c_{\mathrm{O_2}} + \frac{V^{\mathrm{lipid}}}{V^{\mathrm{sample}}} \alpha \left(\frac{c_{\mathrm{SL_m}}}{\kappa} - c_{\mathrm{SL}} \right)$$

$$\frac{\mathrm{d}c_{\mathrm{SLH}}}{\mathrm{d}t} = k_1 c_{\mathrm{SL}} c_{\mathrm{A}} - k_2 c_{\mathrm{SLH}} c_{\mathrm{O_2}} + \frac{V^{\mathrm{lipid}}}{V^{\mathrm{sample}}} \beta \left(\frac{c_{\mathrm{SLH_m}}}{\kappa} - c_{\mathrm{SLH}} \right)$$

$$\frac{\mathrm{d}c_{\mathrm{SL_m}}}{\mathrm{d}t} = -\alpha \left(\frac{c_{\mathrm{SL_m}}}{\kappa} - c_{\mathrm{SL}} \right) + k_4 c_{\mathrm{SLH_m}} c_{\mathrm{O_{2m}}} + \rho c_{\mathrm{SL_m}} c_{\mathrm{A}}$$

$$\frac{\mathrm{d}c_{\mathrm{SLH_m}}}{\mathrm{d}t} = -\beta \left(\frac{c_{\mathrm{SLH_m}}}{\kappa} - c_{\mathrm{SLH}} \right) - k_4 c_{\mathrm{SLH_m}} c_{\mathrm{O_{2m}}} \tag{3}$$

$$\frac{\mathrm{d}c_{\mathrm{A}}}{\mathrm{d}t} = -k_1 c_{\mathrm{SL}} c_{\mathrm{A}} - k_3 c_{\mathrm{A}} c_{\mathrm{O_2}}$$

$$\frac{\mathrm{d}c_{\mathrm{O_2}}}{\mathrm{d}t} = -k_2 c_{\mathrm{SLH}} c_{\mathrm{O_2}} - k_3 c_{\mathrm{A}} c_{\mathrm{O_2}} + \frac{V^{\mathrm{lipid}}}{V^{\mathrm{sample}}} \gamma \left(\frac{c_{\mathrm{O_{2m}}}}{\kappa_{\mathrm{O_2}}} - c_{\mathrm{O_2}} \right)$$

$$\frac{\mathrm{d}c_{\mathrm{O_{2m}}}}{\mathrm{d}t} = -\gamma \left(\frac{c_{\mathrm{O_{2m}}}}{\kappa_{\mathrm{O_2}}} - c_{\mathrm{O_2}} \right) - k_4 c_{\mathrm{SLH_m}} c_{\mathrm{O_{2m}}}$$

$$\kappa = \frac{f_t}{1 - f_t} \frac{V^{\mathrm{aqueous\ phase}}}{V^{\mathrm{lipid\ phase}}} \tag{4}$$

where $c_{\mathrm{SL_m}}$, $c_{\mathrm{SLH_m}}$ and $c_{\mathrm{O_{2m}}}$ are the concentrations of TEMPO, its hydroxylamine form and oxygen in the lipid phase, respectively. α, β and γ denote the exchange rates coefficients for SL*, SLH and oxygen between aqueous solution and lipoprotein respectively. The spin probe reduction and oxidation of hydroxylamine in the lipid phase was described by reactions rate coefficients ρ and k_4. Partition coefficient κ is defined by equation (4) as the concentration ratio of the spin probe in the particle monolayer against the aqueous solution. The volumes were calculated from the experimental concentration of the lipoprotein particles and the intensity ratio f_{T} was calculated from the spectrum in Figure 1. From the best fit of the Eqs. (3) to the experimental data we obtained four parameters k_4, α, β and ρ while k_1, k_2 and k_3 were taken from the reduction kinetics in the homogeneous mixture of reactants. The rate constant γ was estimated according to the data from CRC Handbook of Biochemistry.[23] These data are summarised in Table II and presented in Figure 3. It turned out that the rate constant (k_1), describing the reduction of TEMPO in the pure aqueous solution is smaller than ρ, describing the same process taking place in the surface monolayer of both lipoprotein particles. Comparing the two HDL particles it can be noticed that

all free parameters in the simulation of the experimental data are significantly smaller for HDL_3 with respect to HDL_2 particle in framework of the t-test statistics.

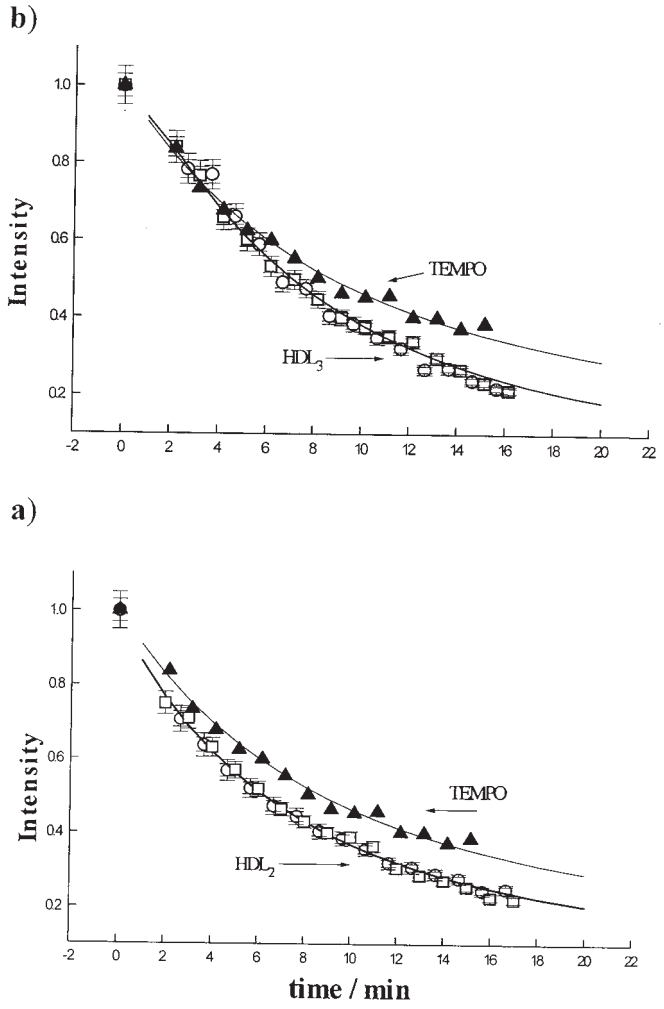

Figure 3. The reduction of TEMPO by ascorbic acid in spin labelled HDL_2 (a) and HDL_3 (b) suspensions.
The following are the concentrations of reactants in the experiments: c(TEMPO) = 0.41 mmol/L, $c(HDL_2)$ = 0.127 mmol/L, $c(HDL_3)$ = 0.334 mmol/L, c(ascorbic acid) = 1.2 mmol/L. Symbols represent experimental ESR intensities, $I(t)$, normalised to the starting intensity, $I(t = 0)$, which are derived as averaged values from the four independent measurements. With the full lines the result of the theoretical simulation according to Eqs. (2) and (3) are shown.

TABLE II

The rate constants used in the simulation of the experimental data

	HDL_2	HDL_3
Rate constant		
k_1/(L / mol min) [a]	90 ± 10	90 ± 10
k_2/(L / mol min) [a]	90 ± 10	90 ± 10
k_3/(L / mol min) [b]	0.01	0.01
k_4/(L / mol min) [c]	150 ± 10	30 ± 7
α/(1/min) [c]	150 ± 50	70 ± 30
β/(1/min) [c]	150 ± 50	70 ± 30
γ/(1/min) [b]	20	20
κ	7.8	8.1
κ_{O2}	4	4
ρ/(L / mol min) [c]	220 ± 6	170 ± 6

[a] Determined in the framework of the model described by Eqs. (2).
[b] Acording to the data from CRC Handbook of Biochemistry.[23]
[c] Determined in the framework of the model described by Eqs. (3).

DISCUSSION

We have studied the reduction of spin probe TEMPO with ascorbic acid in lipoprotein solutions.

The main result of our experiments indicates that in the presence of HDL_2 and HDL_3 particles the reduction rate of the nitroxide is enhanced as compared to the control process without the particles. During the experiment the partition of TEMPO between the lipoprotein particles and the aqueous solution remained unchanged.

To understand the observed phenomenon we applied the mathematical modeling of the complex reaction kinetics taking place in the heterogeneous biological system.

The possibility that HDL itself reduces TEMPO and accelerates the disappearence of ESR spectra had to be excluded since there was no reduction in the labeled HDL in absence of ascorbate.

Therefore, we considered that the overall kinetics results from a series of parallel coupled processes, taking place in the two environments (polar/nonpolar), characterised by specific rate constants. The fact that the partition coefficient stays unchanged during the reduction in both lipoproteins supports the assumption of a fast spin probe exchange between the aqueous solution and the particles.

Undoubtedly, the observed enhanced reduction kinetics in HDL is a consequence of high reduction rates in the hydrophobic environment of the lipid phase. One possible explanation of this phenomenon is the way of insertion of TEMPO molecule into the surface monolayer. We assume that the nitroxide group of TEMPO molecule is facing preferentially the aqueous phase. This convenient orientation could increase the efficiency of encounters with ascorbate. Besides, the positively charged lipoprotein surface might induce a local concentration increase of ascorbate anion at the surface of the particle.

The higher values of the oxidation rate constants of reactions taking place in the HDL_2 as compared with the oxidation in the aqueous phase might also be due to the higher oxygen solubility in the hydrophobic reaction matrix.

We have also observed remarkable differences in the rate constants of reactions taking part in the HDL_2 and HDL_3 particles (Table II). Both, the reductions as well as the oxidation rate constants are larger for the HDL_2 particles. This observation can be confronted with the difference in the curvature of the surface and the content of the unsaturated fatty acids of the monolayer. The smaller HDL_3 particle monolayer exhibits larger curvature and has a higher content of the unsaturated fatty acids (Table I). Therefore, we may assume that it is more exposed to the polar surroundings and thus imposes less structural restrictions for the reaction kinetics to proceed.[24,25]

Comparing α and β coefficients for HDL_2 and HDL_3 a twofold faster process in HDL_2 than in HDL_3 can be deduced (Table II). The explanation of this phenomenon should be searched in the specificity of apoprotein/phospholipids intermolecular forces. Besides, the depth of the hydration layer is determined by the content of unsaturated fatty acids and cholesterol. In HDL_2 the fraction of unsaturated fatty acids is smaller than in HDL_3 and distributed mainly in the core (Table I). On the other hand, the high content of unsaturated FA and protein in the HDL_3 monolayer results in smaller hydrophobic barriers.[26] The interface between the particle's monolayer and the aqueous solution is therefore for HDL_3 characterised with less effective reactivity, which might in part relay on the deeper penetration of water molecules into the monolayer by which the ordering dependent dynamic structure is perturbed.

CONCLUSION

HDL particles are involved as the carriers of hydrophobic molecules in the systemic circulation. The autooxidant activity or interactions of transported molecules with other oxidant substances could be dramatically en-

hanced either *via* local concentration increase or by their exposure on the lipoprotein surface what enables the reaction.

We have observed that the solubilisation of the ambivalent molecule TEMPO in the hydrophobic environment of HDL can enhance its reaction kinetics. From the observed results we can propose that HDL_2 subfraction is more efficient than HDL_3 in promoting the reaction what could be related to the difference in atherogenetic protection behaviour between these two members of the lipoprotein particles family.

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SAŽETAK

Istraživanje kinetike lipidne faze u HDL s pomoću ESR spektroskopije

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Dvije subfrakcije lipoproteina velike gustoće HDL_2 i HDL_3 označene su sa spinskom probom TEMPO koja ulazi i u vodenu i u lipidnu fazu. Dinamika lipidne faze proučavana je praćenjem redukcijskih procesa ugrađene probe TEMPO askorbinskom kiselinom. Redukcija paramagnetnog nitroksida u neparamagnetni hidroksilamin uzrokuje vremensko smanjenje ESR signala. Krivulja redukcije upućuje na složeni proces, pri čemu particijski koeficijent probe TEMPO ostaje nepromijenjen tijekom reakcije. Redukcijski procesi u otopini HDL brži su od redukcijskih procesa u čistim otopinama spinske oznake i askorbinske kiseline. Predložen je model redukcije TEMPO s askorbinskom kiselinom u otopinama HDL. Pretpostavljeno je da su procesi, koji se istovremeno događaju u tom složenom sistemu, određeni ukupnom brzinom reakcije koja ovisi o lokalnim koncentracijama reaktanata kao i o transportnim osobinama čestica u dotičnoj fazi.