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A Spin-Probe Study of the Effect of Sodium Hypochlorite on Human Blood Lipoproteins

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It is well known that lipid peroxidation (LPO), the process generally involving serum lipoproteins (LP), accompanies the development of a variety of pathological processes, in particular atherosclerosis [8,13]. The role of oxidative modifiers can be assigned primarily to reactive oxygen forms such as H_2O_2, $O^{\cdot}_2$, $OCl^{\cdot}$, and $OH^{\cdot}$, which are generated by activated neutrophils and monocyte-derived macrophages [4]. Recently, it was shown that hypochlorite (OCl^{-}) intensively oxidizes lipids and damages the serum LP proteins [1,6,7], thus being a possible cause of the accumulation of modified LP in the human organism.

In the present study we investigated the structural alterations induced by sodium hypochlorite (NaOCl) in the surface proteolipid layer of low-density LP (LDL) from human blood.

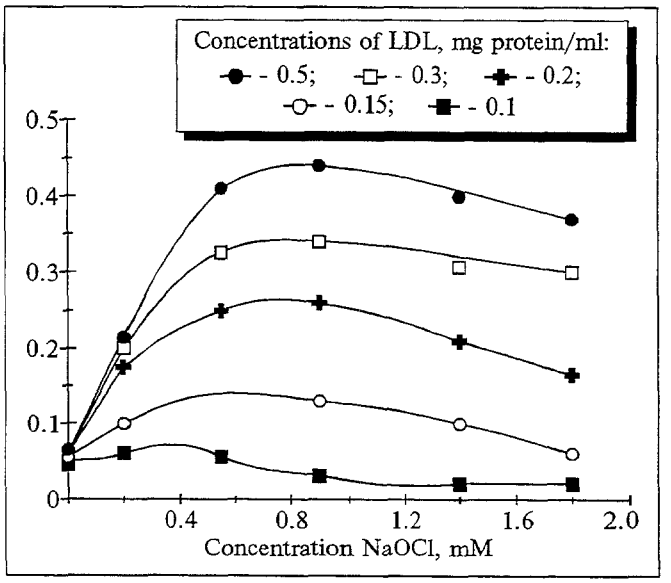

Fig. 1. MDA content (ordinate, μM) as a function of concentration of NaOCl after incubation of LDL with NaOCl at 37°C for 1 h. The incubation medium contains 145 mM NaOCl, 10 mM phosphate buffer saline, pH 7.4.

Research Institute of Physicochemical Medicine, Moscow (Presented by Yu. M. Lopukhin, Member of the Russian Academy of Medical Sciences)

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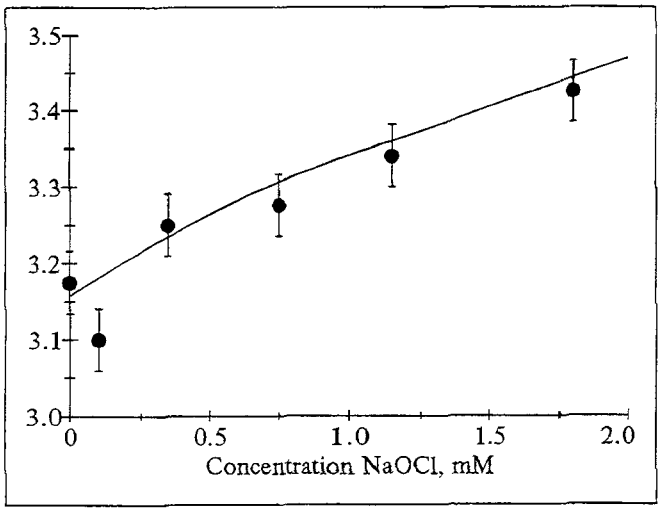
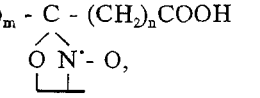


Fig. 2. Correlation time of rotational diffusion (τ, nsec) of C_0 probe in LDL preincubated with NaOCl at 37°C for 1 h as a function of concentration of sodium hypochlorite. Here and in Figs. 3–6: the incubation medium contains 145 mM NaOCl, 10 mM phosphate buffer saline, pH 7.4; concentration of LDL 0.75 mg protein/ml, concentration of probe 10^{-5} M.

MATERIALS AND METHODS

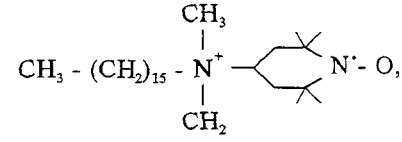
LDL were isolated from human serum by preparative ultracentrifugation in a certain NaBr density as described earlier [9]. The concentration of LDL was determined by measuring the protein content after Lowry [10]. Sodium hypochlorite was obtained from a 0.9% NaCl solution using an EDO-3 apparatus at a constant current of 1A for 30 min. The concentration of NaOCL was determined by its adsorption at 290 nm and pH 12, assuming the molar extinction to be 350 $mol^{-1} \times cm^{-1}$ [12]. The content of 2-thiobarbituric-acid-reactive LPO products (mainly malonic dialdehyde, MDA) was measured as described elsewhere [1].

EPR spectra were recorded with an ER-240 radiospectrometer (Bruker, Germany) at 20°C using the following operation mode: 10 mW SHF radiation power, 0.1 mT HF modulation amplitude, 2.5 mT/min scan time, and time constant 0.3 sec. Paramagnetic analogs of stearic acid with radical linked to the 5th, 12th, and 16th C atoms (5-, 12-, and 16-doxyl stearate, respectively, Sigma, USA):



Spin probe	C_5	C_{12}	C_{16}
m	12	5	1
n	3	10	14

which are localized in the lipid areas in such a way that the carboxyl group is exposed to the polar aqueous phase and the fatty acid chain is positioned in parallel with the acyl chains of phospholipids [2,15]. This allows us to obtain information on the structural organization of LP at different distances from the lipid-water interface. A spin label C_0 (Laboratory of Special Reagents, Bulgarian Academy of Sciences):



represents an amphiphilic molecule with a charged moiety at the end of the hydrophobic hydrocarbon chain, which is also incorporated in the lipid phase, but its paramagnetic center remains on the surface, within the polar groups of phospholipids [11].

LDL were preincubated at 37°C for 1 hour in the presence of 0-1.8 mM NaOCl in 145 mM

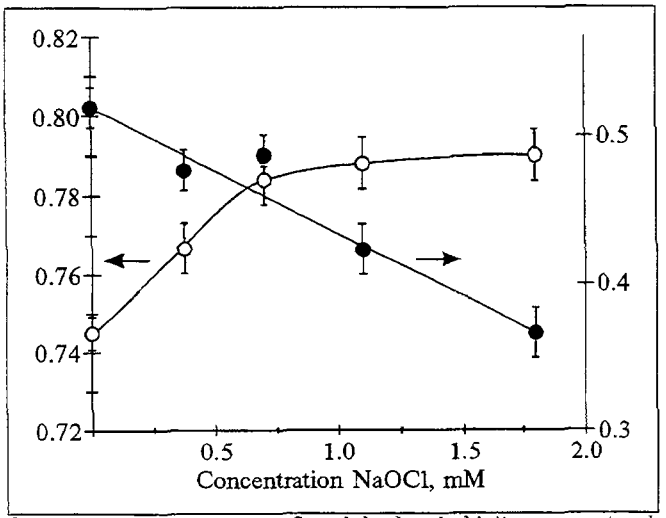


Fig. 3. Order parameter S and hydrophobicity parameter h of the C_5 spin probe in LDL preincubated with NaOCl at 37°C for 1 h as a function of concentration of sodium hypochlorite.

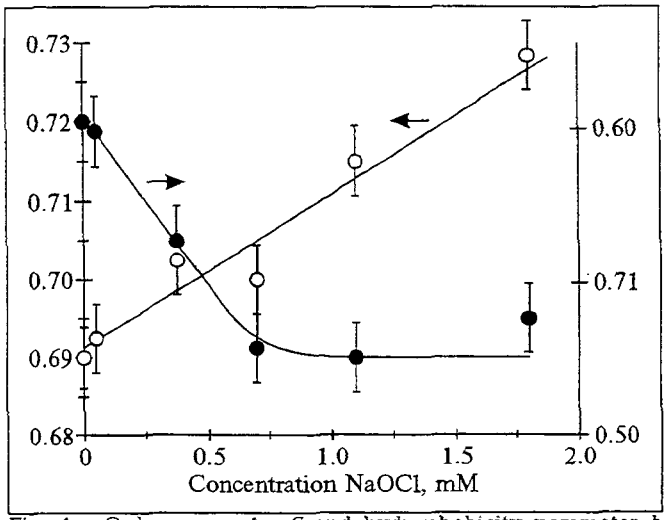


Fig. 4. Order parameter S and hydrophobicity parameter h of the C_{12} spin probe in LDL preincubated with NaOCl at 37°C for 1 h as a function of concentration of sodium hypochlorite.

NaCl, 10 mM phosphate buffer saline, pH 7.4. Then, a spin probe dissolved in ethanol was added to the LDL suspension. The concentration of the probe in the sample was 10^{-5} M, of LDL 0.75 mg protein/ml, and of ethanol 1 vol.%. During the analysis of the EPR spectra of the C_0, C_5, C_{12}, and C_{16} probes inserted in LDL, the following parameters were determined [3]: the order parameter S, the correlation time of rotational diffusion of the nitroxyl moiety τ, and the hydrophobicity parameter h, characterizing the hydrophobicity of the nitroxyl moiety surrounding:

$$h=(a_A-a)/(a_A-a_{OK}),$$

where a, a_A, and a_{OK} are the isotropic constants of superfine interaction of the probe in LDL, aqueous phase, and octanol, respectively. A higher h value corresponds to a more hydrophobic surrounding of the paramagnetic center of the probe [3].

RESULTS

Figure 1 shows the dependences of the MDA content in the incubation medium on the concentration of NaOCl at different LDL concentrations. As seen from the figure, the incubation at 37°C for 1 hour results in the accumulation of 2-thiobarbituric-acid-reactive products of LPO. The curves are bell-shaped and the MDA level rises at low concentrations and declines at higher concentrations of NaOCl, since MDA is an intermediate product in the reaction of hypochlorite-induced oxidation of lipids [1]. Hence, the MDA content cannot serve as a proper criterion of the degree of NaOCl-induced LPO. For this reason, in our subsequent experiments we recorded the changes of spectrum parameters of spin-labeled LDL at various concentrations of NaOCl in the incubation medium.

Using the C_0, C_5, C_{12}, and C_{16} probes, we investigated the structural changes at different depths, from the surface of LDL particles preincubated with NaOCl. As the concentration of NaOCl in the incubation medium increases and LPO products accumulate, we observe an increase of the correlation time of rotational diffusion (τ) of the C_0 probe (Fig. 2). This suggests the restriction of rotational motion of the nitroxyl moiety, localized in the polar surface area of the LDL particle, which is likely to be due to immobilization of the polar groups of phospholipids.

Figures 3 and 4 show the dependences of the order parameter (S) and hydrophobicity parameter (h) of the C_5 and C_{12} probes in LDL on the NaOCl concentration in the incubation medium.

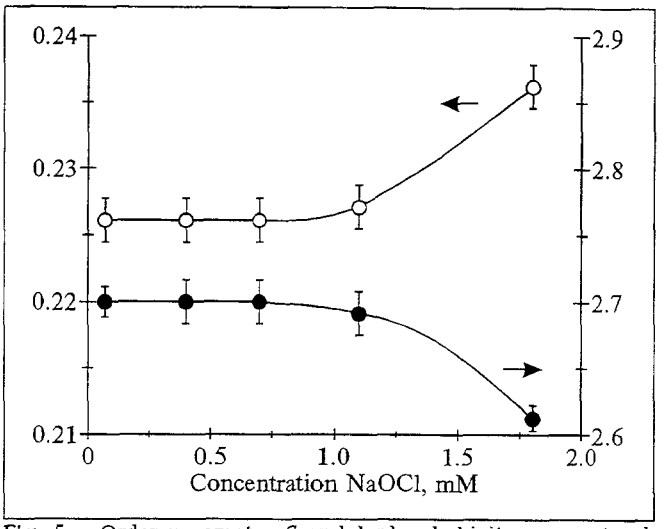


Fig. 5. Order parameter S and hydrophobicity parameter h of the C_{16} spin probe in LDL preincubated with NaOCl at 37°C for 1 h as a function of concentration of sodium hypochlorite.

The increase of the NaOCl concentration led to a rise of parameter S for both the C_5 (Fig. 3) and C_{12} (Fig. 4) probes. In parallel with the rise of parameter S, a decrease of hydrophobicity parameter h was observed for the C_5 and C_{12} probes. This implies that NaOCl-induced LDL oxidation leads to a simultaneous increase in the order and a decrease in the hydrophobicity of fatty-acid chains of the C_5 and C_{12} probes within the 5th-12th CH_2 groups (i.e., at a depth of approximately 0.5-1.7 nm from the LDL surface).

For the C_{16} probe we also recorded an increase of parameter S and a decrease of parameter h with the NaOCl concentration increase (Fig. 5). However, these changes occurred only at NaOCl concentrations of more than 1 mM. In this case no reliable change of parameter τ was detected (Fig. 6).

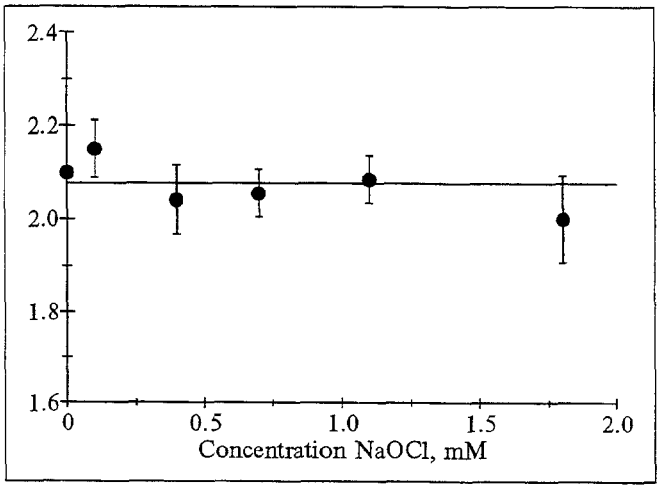


Fig. 6. Correlation time of rotational diffusion (τ, nsec) of C_{16} probe in LDL preincubated with NaOCl at 37°C for 1 h as a function of concentration of sodium hypochlorite.

From the data obtained the conclusion may be drawn that NaOCl induces lipid peroxidation in LDL, which leads to disturbances in the structure of the surface proteolipid layer of the particles. This manifests itself, on the one hand, as restricted mobility (an increase of parameters S and τ), and, on the other, as an increased polarity (drop of parameter h) of the microenvironment of fatty-acid chains of phospholipids as deep as the 12th CH_2 group (approximately 1.7 nm from the lipid-water interface). Marked changes in deeper regions (16th CH_2 group) become detectable only at concentrations of NaOCl exceeding 1 mM. It should be noted that earlier we observed similar qualitative changes in the mobility and polarity of the phospholipid acyl chains depending on the degree of LDL autooxidation [5,13,14].

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Behavioral Effects of β-Casomorphin-7 and Its Des-Tyr-Analogs

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Early investigations of the effects of opioid peptides on behavior and the exploratory reaction demonstrated that intracerebral administration of morphine, endorphins, and Met-enkephalin causes muscular rigidity and immmobility in experimental animals [4,16]. Further studies revealed a more complex character of opioid influence. Depending on the type of peptide, the mode of its administration into the organism, the species of experimental animal, and the peptide dose, it is possible to register an entire spectrum of effects, ranging from significant motor excitation to a complete inhibition of locomotor activity and catotonia [14].

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