2. N. V. Gulyaeva, *Lipid Peroxidation in the Brain during Adaptation to Stress* [in Russian], Ph.D.Dissertation, Moscow (1989).
3. N. V. Gulyaeva, *Nauchn. Dokl. Vyssh. Shkol., Biol. Nauki.*, № 4, 8-14 (1989).
4. M. L. Libe, V. I. Brusovanik, L. Yu. Ukhin, *et al., Byull. Eksp. Biol.*, **112**, № 7, 11-12 (1991).
5. F. Z. Meerson, in: *Physiology of Adaptative Processes* [in Russian], Moscow (1986), pp. 10-76.
6. J. Folch, M. Lees, and G. H. Sloan-Stanley, *J. Biol. Chem.*, **226**, № 3, 447-453 (1957).
7. H. Ohkawa, N. Ohishi, and K. Yagi, *Analyt. Biochem.*, **95**, № 2, 351-358 (1979).
8. N. Osborn, *Microchemical Analysis of Nervous Tissue*, New York (1974), pp.171-200.
9. G. Scatchard, *Ann. N.Y. Acad. Sci.*, **51**, 660-672 (1949).
10. V. P. Wittaker, in: *Handbook of Neurochemistry*, Vol. 7, New York-London (1984), pp.1-39.

The Ability of Sodium Hypochlorite to Penetrate the Lipoid Phase

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Lipoproteins (LP) in human blood can be modified as a result of lipid peroxidation (LPO). It has been suggested that the accumulation of such modified LP in the blood can cause atherosclerosis [9,13]. LP in the blood are oxidized by $O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$, and OCl^- formed during the activation of phagocytes [5]. In a recent study we have shown [6] that NaOCl can oxidize lipids that form part of the LP in human blood. This reaction takes place with the participation of free radicals and ions of transition metals [2,6].

It is known that LPO takes place at the site of unsaturated bonds in the fatty acid chain, i.e., in the hydrophobic hydrocarbon portion of the lipid phase of LP and in biological membranes [1]. But in that case it is not quite clear in what way NaOCl participates in LPO in lipoproteins, since NaOCl is readily soluble in water and is localized in all probability in the aqueous phase.

The present work is an attempt to ascertain whether sodium hypochlorite penetrates into the lipid phase of protein-lipid complexes. For this purpose NaOCl was introduced into an aqueous suspension of low-density lipoprotein (LDL) isolated from human blood, and the reaction of NaOCl with the electron donors localized in the lipid phase of LDL was investigated. Used as electron donors were nitroxide radicals which are derivatives of stearic acid with a paramagnetic center localized at various distances from the carboxyl group.

MATERIALS AND METHODS

LDL was isolated from human blood serum by ultracentrifugation of a NaBr solution of a certain density containing the serum [10]. The concentration of LDL was determined from the protein content in LDL by the Lowry method [11]. Sodium hypochlorite was prepared electrochemically with the aid of an EDO-3 setup by passing a direct current (1 amp) for 30 min through a 0.9% solution of NaCl. The concentration of NaOCl was determined at pH 12 from the absorption at 290 nm; the molar extinction coefficient of NaOCl was assumed to be 350 $mol^{-1}cm^{-1}$ [12].

The EPR spectra of the spin probes were recorded at 20°C with the aid of an ER-420 radio-

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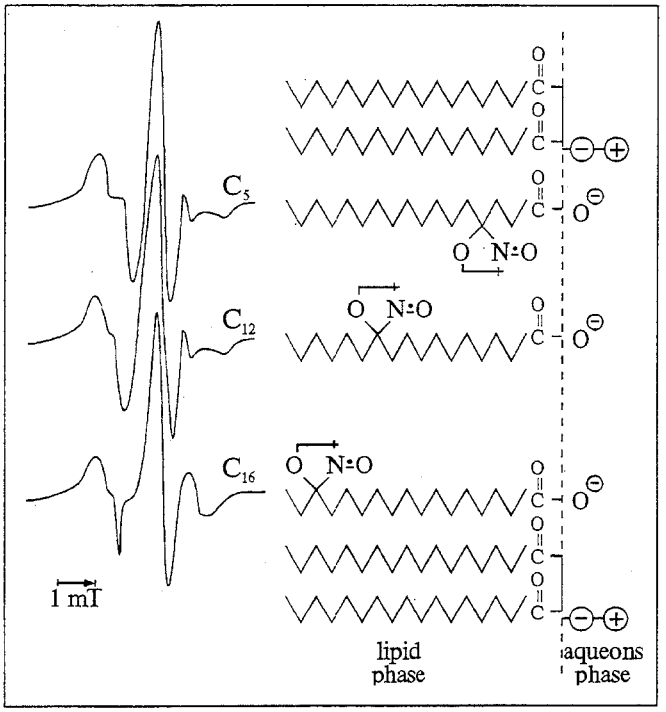

Fig. 1. EPR spectra, structural formulas, and a diagram showing C_5, C_{12}, and C_{16} spin probes in the lipid surface layer of LDL.

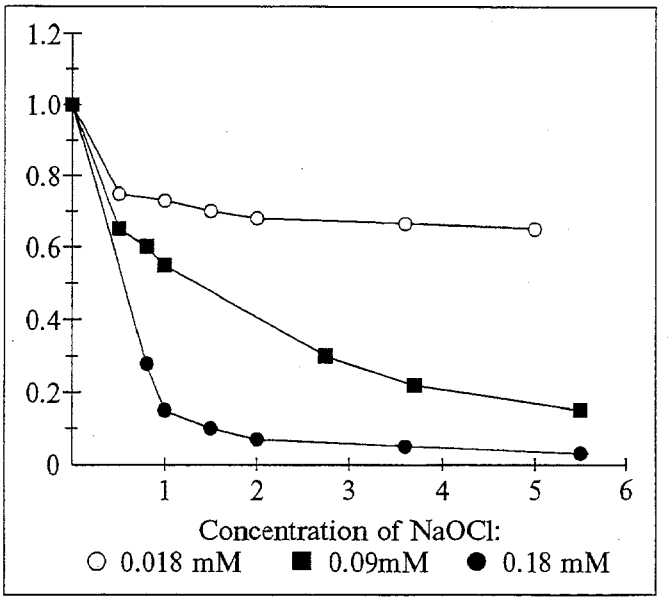

Fig. 2. Kinetic curves (in min) for the oxidation of the C_5 spin probe (10^{-5} M) at different concentrations of aqueous NaOCl: 145 mM NaCl solution; 10 mM phosphate buffer; pH 7.4; temperature 20°C; h_o and h_t are the amplitudes of the central components in the EPR spectrum before the introduction of NaOCl and t minutes after it, respectively.

spectrometer (Brucker, Germany) under the following conditions: ultra-high frequency power 10 mW; modulation amplitude 0.1 mT; rate of magnetic field scanning 2.5 mT/min at a time constant of 0.3 sec. The spin probes used were paramagnetic analogs of stearic acid with a radical fragment of the 5th, 12th, and 16th C-atom (5-, 12-, and 16-doxyl stearate, respectively), henceforth referred to as C_5, C_{12}, and C_{16} (Sigma, USA). The samples were prepared by adding 1 μl of an ethanol solution of the probe (the final concentration of the probe was 10^{-5} M, and of the ethanol solution 1% by vol.) to 100 μl of an LDL suspension in a solution (pH 7.4) containing 145 mM NaCl and 10 mM phosphate buffer. The mixture was stirred, and the first EPR spectrum was recorded. Following this, 1 μl of a NaOCl solution was added to the probe, and the central line in the EPR spectrum was registered every 20-60 sec.

The oxidation kinetics for the probe was determined from the decrease in the amplitude of the central EPR component as a function of time. The results obtained were used to plot the kinetic changes in parameter h_t/h_o (where h_o and h_t are the amplitudes of the central EPR signal registered before the introduction of NaOCl and t minutes after it, respectively).

RESULTS

It is known [3,14] that in model and in natural protein-lipid complexes the spin probes (paramagnetic analogs of stearic acid) are localized in lipids in such a way that the carboxyl group lies in the polar aqueous phase. The fatty acid chains are suspended in the hydrophobic phase and lie parallel to the acyl chains of the phospholipids (Fig. 1). This makes it possible to determine the physicochemical properties of the object under consideration at various distances from the lipid-water interface.

Nitroxyl radicals are capable of undergoing oxidation, which leads to the formation of diamagnetic salts of ammonium hydroxide [7]. As can be seen

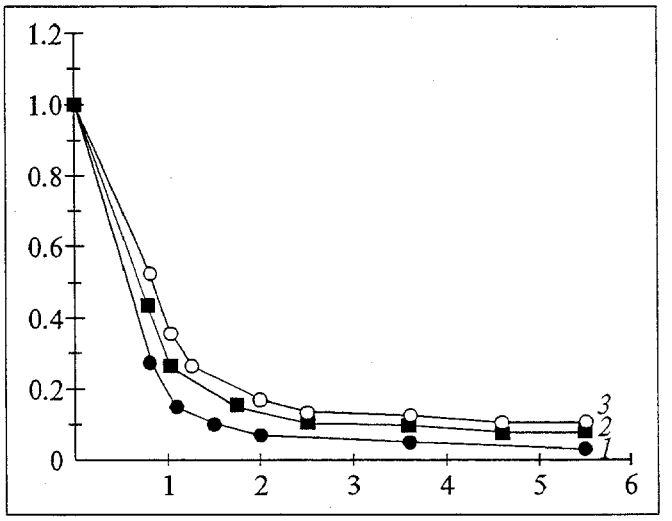

Fig. 3. Kinetic curves (in min) for the oxidation of the C_5 (1), C_{12} (2), and C_{16} (3) spin probes in the presence of aqueous NaOCl: 145 mm NaCl solution; 10 mM phosphate buffer; pH 7.4; temperature 20°C; concentration of initial probe and of NaOCl 10^{-5} M and 0.018 mM, respectively.

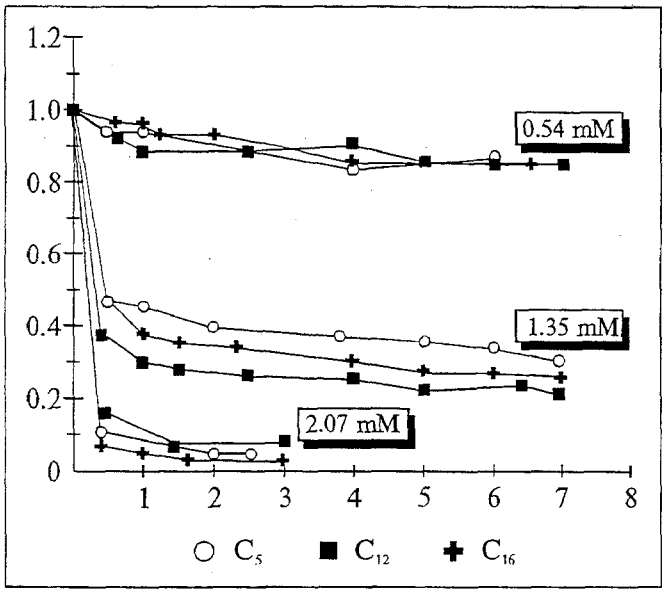

Fig. 4. Kinetic curves (in min) for the oxidation of the C_5, C_{12}, and C_{16} spin probes implanted into LDL in the presence of aqueous NaOCl. Temperature 20°C; concentration of the probe 10^{-5} M; concentration of LDL 0.85 mg protein/ml. Figures indicate NaOCl concentration.

from Fig. 2, the C_5 spin probe was oxidized when NaOCl was added to its aqueous solution. The rate of the oxidation reaction increased with an increase in the concentration of NaOCl. In Fig. 3 are shown kinetic plots describing the oxidation of C_5, C_{12}, and C_{16} spin probes with aqueous NaOCl. As can be seen, the rate of oxidation of all three probes was the same (within the limits of experimental error).

To find out whether NaOCl penetrates into the lipid phase of LP an investigation was conducted of the oxidation of the C_5, C_{12}, and C_{16} spin probes with aqueous NaOCl, the probes being inside the phospholipid surface monolayer of LDL. Figure 4 presents kinetic plots describing the oxidation of the C_5, C_{12}, and C_{16} spin probes with an NaOCl suspension in LDL. As can be seen, all three probes deposited on LDL became oxidized, and the rate of oxidation increased with an increase in the concentration of the NaOCl added.

Our measurements showed that all the probes were oxidized at the same rate. This is contrary to numerous data reported in the literature [3,14] indicating that the oxidation proceeds at an increasing rate in the order C_5 - C_{12} - C_{16}, depending on the depth to which the probe's nitroxyl fragment penetrates the lipid phase. This contradiction is understandable if we remember that the pH of hypochlorous acid (HOCl) is 7.5 [12]; in other words, when the pH values are at physiological levels, about half of NaOCl is in an nonionized state. It is known that NaOCl molecules can penetrate biological membranes [4,15]. Apparently, the penetration of NaOCl into the area of localized spin probes in LDL has no limiting effect on the oxidation process in question. However, as can be seen from Fig. 4, the oxidation of the probes with NaOCl inside LDL is of a biphasic nature, unlike the oxidation in the aqueous phase (Fig. 3). There may be two explanations for this. Firstly, it is possible that some of the paramagnetic centers of all three probes are only slightly accessible or not accessible at all to NaOCl. Secondly, as oxidation proceeds a part of the NaOCl is consumed in reactions with other components of the system (first of all, in the reaction with the SH and NH_2 groups in the proteins). The latter explanation is the more likely, since it is known that NaOCl readily reacts with SH and NH_2 groups in the apolipid of LDL [8].

Thus, by using spin-labeled analogs of stearic acid, we obtained results which indicate that NaOCl can penetrate into the surface phospholipid layer of LDL, oxidizing at the same rate spin probes localized at various distances from the lipid-water interface. This probably explains the high efficiency of NaOCl in initiating LPO in LP, as was observed in our previous investigations [2,6].

REFERENCES

1. Yu. A. Vladimirov and A. I. Archakov, *Lipid Peroxidation in Biological Membranes* [in Russian], Moscow (1972).
2. S. A. Evgina, O. M. Panasenko, V. I. Sergienko, and Yu. A. Vladimirov, *Biol. Membrany*, 9, № 9, 946-953 (1992).
3. I. Zelig, in: *Spin Labeling: Theory and Applications*, ed. L. J. Berliner, Academic Press (1976).
4. V. V. Lukashov, in: *Electrochemical Methods in Medicine* [in Russian], Moscow (1991), pp. 9-10.
5. A. N. Mayanskii and D. N. Mayanskii, *Essays on the Neutrophil and Macrophage* [in Russian], Novosibirsk (1989).
6. O. M. Panasenko, S. A. Evgina, and V. I. Sergienko, in: *Electrochemical Methods in Medicine*, op. cit., pp. 7-8.
7. E. G. Rozantsev, in: *Free Iminooxide Radicals* [in Russian], Moscow (1970), p. 83.
8. J. Arnold, S. Hammerschmidt, M. Wagner, *et al.*, *Biomed. Biochim. Acta*, **49**, 991-997 (1990).
9. J. Heinecke, *Free Rad. Biol. Med.*, **3**, 65-73 (1987).
10. F. T. Lindgren, in: *Analysis of Lipids and Lipoproteins*, ed. E. G. Perkins, Champaign (Ill.) (1975), pp. 204-224.
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
12. J. C. Morris, *J. Phys. Chem.*, **70**, 3798-3805 (1966).
13. O. M. Panasenko, O. A. Azizova, and Yu. A. Vladimirov, *Sov. Med. Rev. Sect. B. Physicochem. Asp.*, **3**, Pt. 3, 1-74 (1992).
14. S. Schreier-Muccillo, D. Marsh, and I. C. P. Smith, *Arch. Biochem.*, **172**, 1-11 (1976).
15. S. Weiss and A. F. Lobuglio, *Lab. Invest.*, **47**, № 1, 5-18 (1982).