Role of Myeloperoxidase-Mediated Modification of Human Blood Lipoproteins in Atherosclerosis Development

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The mechanism of interaction of hypochlorite and hypobromite formed in myeloperoxidase catalysis with lipids of human blood low-density lipoprotein is described. Both agents react with unsaturated lipids via two mechanisms: molecular (with the formation of mainly chloro- or bromohydrins and lysophospholipids) and free-radical (paralleled by lipid peroxidation). These reactions modify physicochemical properties of low-density lipoproteins and disorder their lipid-transporting function thus initiating early stages of atherosclerosis development.

Key Words: *low-density lipoproteins; myeloperoxidase; atherosclerosis; hypochlorite; free radicals*

One of the main clinical manifestations of atherosclerosis is accumulation of lipids, mainly cholesterol esters, in the arterial wall. The so-called modified LDL play an important role in this process. In 1980s several research groups revealed and isolated from the blood of atherosclerosis patients an LDL subfraction, which differed significantly from native LDL by its physicochemical characteristics [12,17, 25]. These modified LDL promoted accumulation of intracellular cholesterol and formation of early atherosclerotic lesions in the arterial intima.

The cause of LDL modification *in vivo* remains not clear. Several enzyme systems detected in atherosclerotic vessels, primarily myeloperoxidase (MPO), are the modifier candidates. Myeloperoxidase is present in neutrophils and monocytes, is secreted into extracellular medium during activation of these cells, and catalyzes oxidation of halides (Cl^-, Br^-, I^-) and pseudohalides (SCN^-) to highly reactive hypohalogenites (HOX/OX^-) [15,16]:

$$H_2O_2+X^-+H^+\rightarrow HOX+H_2O$$
$$OX^-+H^+\leftrightarrow HOX$$
$$(X=Cl, Br, I, SCN). \quad (1)$$

The resultant hypohalogenites, among which hypochlorite ($HOCl/OCl^-$) and hypobromite ($HOBr/OBr^-$) deserve special attention, are strong oxidizers reacting with all compounds in LDL: protein, phospholipids, cholesterol, carbohydrates, antioxidants (carotenoids, α-tocopherol) [1,6,19,21].

The enzyme [14] and significantly elevated content of hypochlorite-modified protein [18] were detected in human vessels and other tissues involved in atherosclerosis.

During the recent 15 years our efforts were focused on studies of the mechanism of modification of LDL lipids under the effects of $HOCl/OCl^-$ and $HOBr/OBr^-$, as well as of systems producing these agents ($MPO+H_2O_2+Cl^-/Br^-$, activated neutrophils). Based on our findings, two main mechanisms of lipid impairment by hypohalogenites are distinguished.

The molecular mechanism is realized without participation of free radicals. One of reactions realized by this mechanism is hypochlorite reaction

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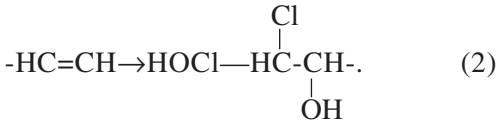
with unsaturated bonds of free fatty acids and fatty-acid chains of phospholipids, triglycerides, and cholesterol esters:



-HC=CH→HOCl—HC(Cl)-CH(OH)-. (2)

Hypobromite reacts similarly. Chlorohydrin isomers (bromohydrin isomers in the reactions with hypobromite) are formed in this reaction. Hypohalogenites react with all unsaturated bonds in the fatty-acid chain [5,9,10,22,24]. Lysophospholipid was the main product of reactions of polyunsaturated phospholipids, containing arachidonic or docosahexaenoic acid residues, with hypohalogenites or with MPO+H_2O_2+Cl^-/Br^- system. The more unsaturated were acyl chains in phospholipids, the more rapid and intense was lysophospholipid accumulation in them [5,9,10,22].

Let us see how polyunsaturated phosphatidylcholine transforms into a lysoderivative after reaction with HOCl/OCl^- or HOBr/OBr^-. Introduction of such electron acceptor substitutes as chlorine, bromine, or hydroxyl into an acyl carbohydrate chain in the synthesis of chloro- or bromohydrins should promote the formation of electron density deficit on the carbon atom of the carbonyl group and, hence, attenuation of the initially weak complex ester bond as a result of appearance of the so-called negative inductive effect (-*I*-effect; Fig. 1). The strength of this effect depends on the quantity and efficiency of electron acceptor substitutes and it rapidly decreases with increasing the number of carbon atoms in saturated chain. Hence, the appearance of chloro- or bromohydrins at the site of double bond in the 4th (for docosahexaenic acid residue) or 5th (for arachidonic acid residue) positions is critical for the formation of lysophospholipids [9,10].

These results indicate that MPO can to a certain measure function as phospholipase, causing (by means of catalytic synthesis of HOCl/OCl^- and/or HOBr/OBr^-) the formation of chloro- and/or bromohydrins, respectively, from unsaturated phospholipids with subsequent spontaneous hydrolysis of their ester bond with the formation of lysophospholipids. This reaction can be biologically important, because lysophosphatidylcholine regulates various pathophysiological processes, for example, exhibits characteristics of an atherothrombotic molecule [23]. It is a chemoattractant for monocytes and T lymphocytes, induces the expression of growth factor and adhesion molecules in endothelial cells, is a mitogen for macrophages and smooth-muscle cells, inhibits mobility of endothelial cells and NO-dependent vascular relaxation, stimulates the production of active oxygen forms by vascular cells through activation of NADPH oxidase [9,23].

Free-radical mechanism of LDL lipid modification with participation of hypohalogenites (as exemplified by hypochlorite) can be as follows (Fig. 2): phagocyte stimulation is paralleled by activation of NADPH oxidase (membrane-bound enzyme) catalyzing the formation of $^{\bullet}O_2^-$. This latter one dysmutates to H_2O_2 spontaneously or under the effect of another enzyme (SOD). During phagocyte activation one more enzyme (MPO) is released into extracellular medium; this enzyme catalyzes the formation of HOCl/OCl^- by reaction (1). Each of the above mentioned active oxygen forms (HOCl/

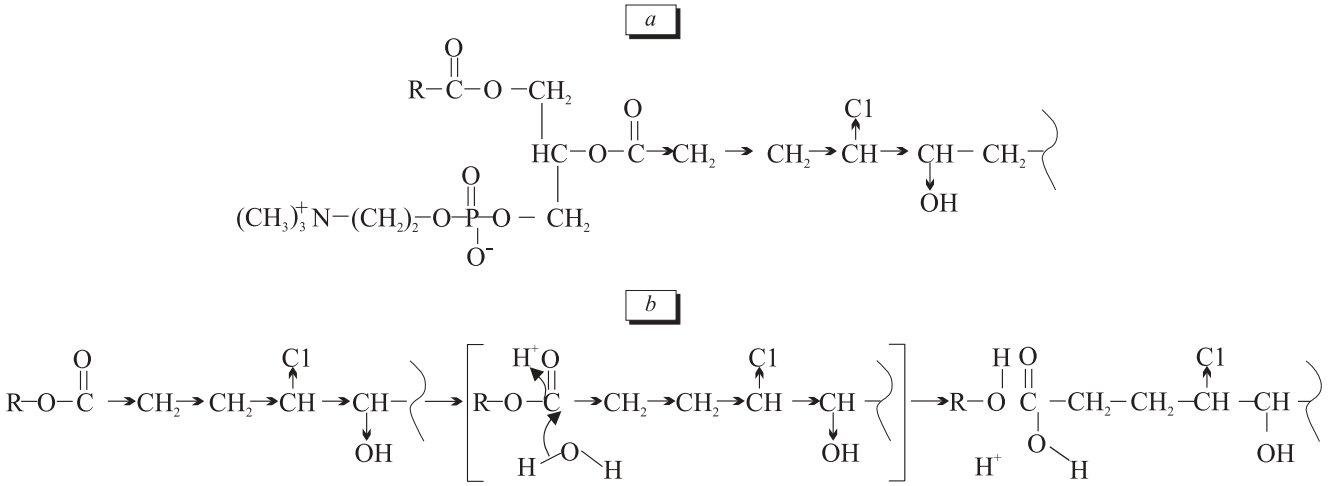


Fig. 1. Scheme of manifestation of the negative inductive effect as a result of chlorohydrin formation in position 4 by the first double bond of phosphatidylcholine docosahexaenoic acid (*a*) and subsequent hydrolysis of complex ester bond with the formation of lysophosphatidylcholine (*b*).

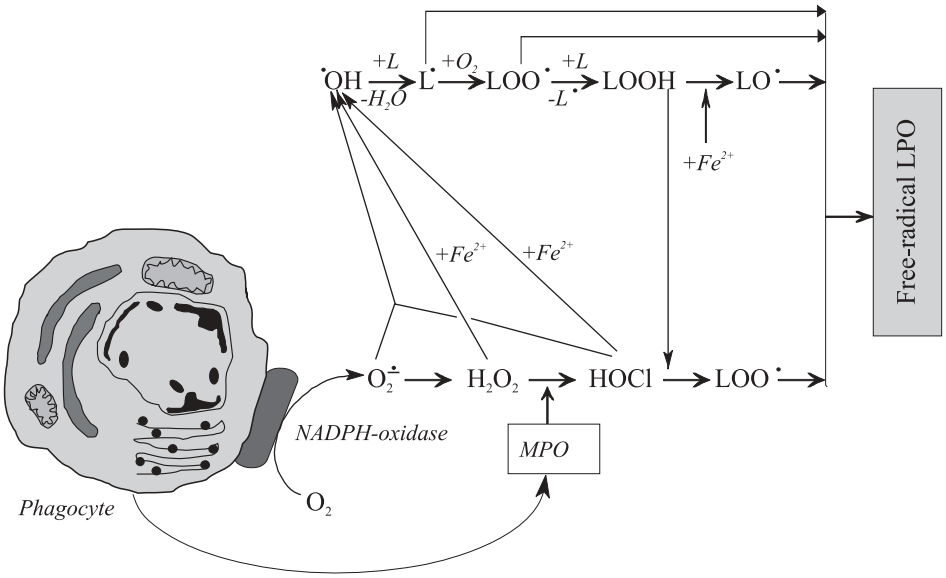


Fig. 2. Scheme of involvement of hypochlorite produced by activated phagocytes, in initiation of free-radical LPO.

OCl^-, $^\bullet O_2^-$, H_2O_2) can become, as a result of subsequent reactions, a source of extremely reactive $^\bullet OH$ radical:

$$HOCl+{}^\bullet O_2^- \rightarrow {}^\bullet OH+O_2+Cl^-$$
$$HOCl+Fe^{2+} \rightarrow {}^\bullet OH+Fe^{3+}+Cl^-$$
$$H_2O_2+Fe^{2+} \rightarrow {}^\bullet OH+Fe^{3+}+OH^-. \quad (3)$$

This radical removing the hydrogen atom from unsaturated lipid (L) forms an alkyl radical ($L^\bullet$; Fig. 2). This latter one can initiate free-radical LPO reactions or can react with O_2 molecule and form a peroxyl radical ($LOO^\bullet$), from which, in turn, a hydroperoxide (LOOH) forms. Hydroperoxide can be reduced by, for example, Fe^{2+} ions to alcoxyl radical ($LO^\bullet$) or can be oxidized by hypochlorite [4,8] or hypobromite [11] to peroxyl radical. Both radicals are LPO initiators. We have shown that hypobromite, though it produces free radicals in reaction with hydroperoxide, but in contrast to hypochlorite, it does not initiate LPO in liposomes from unsaturated phosphatidylcholine under similar conditions [11].

Hence, hypochlorite reaction with hydroperoxide is the most probable candidate for the LPO initiation stage [4,8,20] occurring in hypochlorite reaction with an unsaturated lipid, including blood lipoprotein lipid.

Hypochlorite penetrates into the lipid phase of blood lipoproteins [2]. This leads to degradation of lipid-soluble antioxidants [1,6,21], reduces the resistance of lipid phase to peroxidation [6], increases the content of primary, secondary, and final products of lipid oxidation [1], reduces the mobility and increases the polarity of the lipid phase acyl chains [3].

Complex (molecular and free-radical) modification of LDL induced by hypochlorite impairs their cholesterol-transporting function. They are easier captured by macrophages, which leads to cholesterol accumulation in cells and their transformation into foamy cells, giving rise to atherosclerotic involvement of the vessels [7,19,21].

Which of the mechanisms, molecular of radical, predominates *in vivo* and plays the key role in MPO-induced damage of LDL lipids? Judging from the volume of resultant products and involved functional lipid groups, it is the molecular mechanism, as the concentration of forming chlorohydrins, bromohydrins, or lysophospholipids is significantly higher than the concentration of LPO products. It is however known that LPO reactions significantly modulate the physicochemical properties of the LDL lipid phase and other protein-lipid complexes [1,7]. Therefore, if LPO takes place *in vivo* and plays an important role in the development of many pathological processes, the possibility of hypochlorite initiation of free-radical reactions in foci of inflammation should not be neglected. It becomes obvious, if we recollect that MPO, secreted into extracellular medium during phagocyte activation, associates with protein-lipid complexes, specifically, with blood lipoproteins [13]. Due to this the enzyme can locally realize the oxidative processes in the immediate vicinity of the lipoprotein lipid phase, modifying them and rendering them atherogenic properties.

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