MECHANISMS OF ACTION OF Ca^{++} IONS ON LIPID PEROXIDATION

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Calcium is a universal regulator of many metabolic processes [4, 7, 14]. It has recently been shown that Ca^{++} ions can regulate free-radical lipid peroxidation (LPO) also [1, 2, 12]. Moreover, they cannot only stimulate LPO (in the region of low concentrations of about 10^{-5} M), but they may also exhibit an inhibitory effect (in concentrations of about 10^{-4} M or higher) [2]. The mechanisms of this biphasic effect of Ca^{++} on LPO have not been finally explained, although it has been suggested that the action of high Ca^{++} concentrations on LPO may be based on its interaction with superoxide anion-radicals ($O_2^{\cdot}$) [1, 9, 13].

The aim of this investigation was to study the mechanisms of action of Ca^{++} on LPO in simple model systems (ovolecithin liposomes, emulsions of linolenic acid and methyl linolenate), in which LPO was induced with the aid of an $O_2^{\cdot}$-generating system (Fe^{++} + ascorbate) [5], or with the aid of a system generating peroxide radicals — RO_2 (Fe^{++} + cumyl hydroperoxide) [8].

METHODS

Ovalbumin was obtained by the method in [11]. Linolenic acid $C_{18:3}$ (from Reakhim, USSR; pure) was purified from oxidation products by chromatography on a silica-gel column [3]. Liposomes and ufasomes ($C_{18:3}$ vesicles) were prepared by ultrasonic dispersion of the lipids (1 mg/ml) by means of a UZDN-1 ultrasonic disintegrator with a titanium tip 10 mm in diameter (150 W, 22 kHz, 3·30 sec). LPO in the liposomes and ufasomes was induced in 50 mM phosphate buffer (pH 7.4) at 37°C in the presence of 0.5 mM ascorbate or 1 mM cumyl hydroperoxide, and also of Fe^{++}. On addition of La^{+++} ions 0.05 M Tris-HCl buffer (pH 7.4, 37°C) was used. Accumulation of LPO products was recorded as the formation of malonic dialdehyde (MDA) spectrophotometrically (MPS 50, Shimadzu, Japan) at 535 nm ($\varepsilon = 1.56 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$) [10]. The accumulated MDA (Δ MDA) was determined as the quantity of MDA in the samples at the end of incubation after deduction of the original quantity of MDA.

RESULTS

Kinetic curves of MDA accumulation during auto-oxidation of ovolecithin and linolenic acid, catalyzed by an Fe^{++} + ascorbate system in the presence of different Ca^{++} concentrations, are shown in Fig. 1A. It was found that Ca^{++} has a twofold action on oxidation of lipids: In a concentration of 10^{-5} M it stimulated MDA accumulation, but inhibited it in a concentration of 10^{-3} M. In a more detailed study of dependence of the action of Ca^{++} on concentration in this system the maximal effect of LPO stimulation was observed always when Ca^{++} concentrations in the medium corresponded exactly to the Fe^{++} concentration. In cases when the Ca^{++} concentration exceeded that of Fe^{++}, the stimulating effect of Ca^{++} weakened and gave way to inhibition of LPO (Fig. 1B). A strict linear relationship was found between the Fe^{++} concentration and the Ca^{++} concentration inducing maximal stimulation of LPO (Fig. 2). It can be postulated that the stimulating action of Ca^{++} is based on its ability to interact with negatively charged groups of lipids (phosphate groups of lecithin, carboxyl groups of linolenic acid), thereby displacing the bound Fe^{++} ions [6], i.e., increasing the concentration of free Fe^{++} ions, which participate directly in LPO catalysis. If this hypothesis is true, it will be evident that on induction of peroxidation of the methyl ester of linolenic acid, which has no negatively charged carboxyl groups, no stimulating effect of Ca^{++} ought to be exhibited. In fact it was found that Ca^{++} ions, in the concentration range from 10^{-6} to 10^{-5} M, do not

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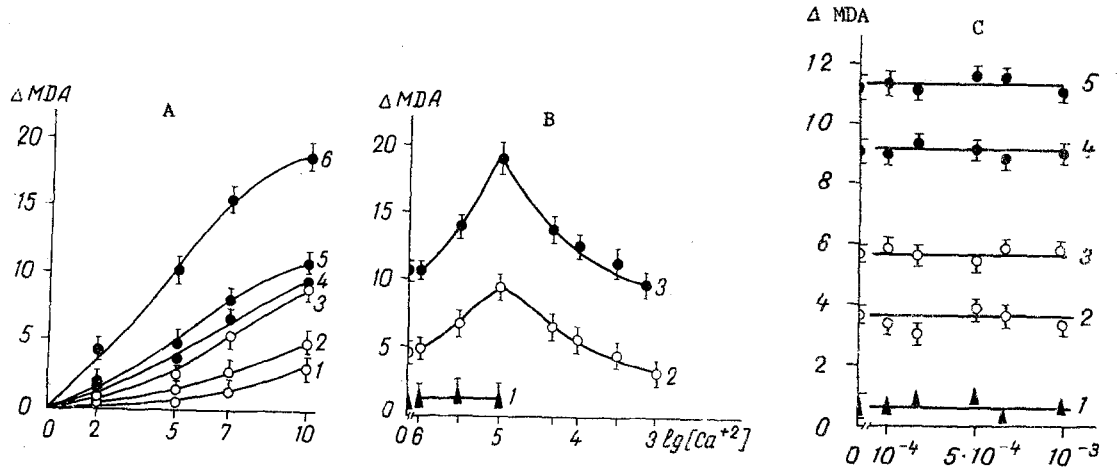

Fig. 1. Action of Ca^{++} on LPO in model systems. Abscissa: A) duration of incubation (in min); B and C) concentration of Ca^{++} added (in M); ordinate, MDA accumulation (in namomoles/mg lipids). A) Oxidation of linolenic acid (1-3) and ovolecithin (4-6), catalyzed by Fe^{++} + ascorbate system (0.06 and 0.5 mM respectively): 2, 5) control; 3, 6) 10^{-5} M Ca^{++}; 1, 4) 10^{-3} M Ca^{++}; B) oxidation of methyl linolenate (1), of linolenic acid (2), and of ovolecithin (3), catalyzed by Fe^{++} + ascorbate system. Incubation time 10 min; C) oxidation of methyl linolenate (1), linolenic acid (2, 3), and ovolecithin (4, 5), catalyzed by Fe^{++} + cumyl hydroperoxide (1 mM) system. Incubation time 10 min; Fe^{++} concentration: 10^{-4} M (1, 3, 5) and 10^{-5} M (2, 4).

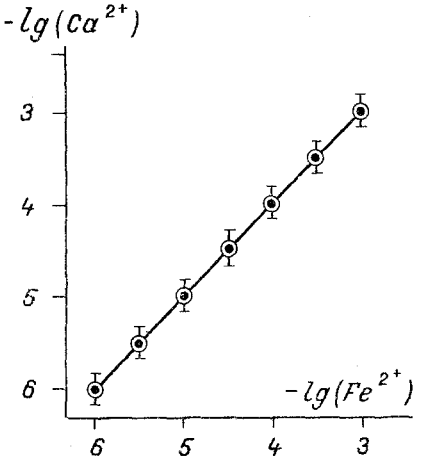

Fig. 2. Correlation between Ca^{++} concentration causing maximal stimulation of LPO induced by Fe^{++} + ascorbate system, and Fe^{++} concentration in incubation medium containing liposomes of ovolecithin (1 mg/ml) or ufasomes of linolenic acid (1 mg/ml).

stimulate oxidation of methyl linolenate in an Fe^{++} + ascorbate system (with a concentration of Fe^{++} of 10^{-5} M).

It was postulated previously that the inhibitory action of high Ca^{++} concentrations on LPO is based on its interaction with $O_2^{\cdot}$ radicals [1, 2]. If this hypothesis is true, in LPO induction systems not dependent on $O_2^{\cdot}$ formation, no inhibitory effect of Ca^{++} ought to be exhibited. Comparison of the results obtained (Fig. 1B, C) shows that whereas high Ca^{++} concentrations had an inhibitory effect in an $O_2^{\cdot}$ dependent system of LPO induction (Fe^{++} +

TABLE 1. Effect of Ca^{++} and La^{++} Ions on MDA Formation during Oxidation of Linolenic Acid by Fe^{++} + Ascorbate System (M ± m)

Ions added	Concentration, M	MDA accumulation, nanomoles/mg fatty acid
Control	0	23,52±2,04
Ca^{2+}	10^{-4}	26,17±1,78
La^{3+}	10^{-4}	30,00±2,18*
$Ca^{2+}+La^{3+}$	$10^{-4}+10^{-4}$	17,91±1,06*

Legend. Incubation medium: 0.05 M Tris-HCl buffer, pH 7.4 (37°C), 0.1 mM Fe^{++}, 0.5 mM ascorbate. Incubation time 7 min. Initial degree of oxidation of linolenic acid 1.88 ± 0.21 nanomoles MDA/mg. *P < 0.01 compared with control.

ascorbate), in an $O_2^{\cdot}$ independent system of LPO induction (Fe^{++} + cumyl hydroperoxide) this inhibitory effect was absent for all the three lipids studied: ovolecithin, linolenic acid, and methyl linolenate.

Incidentally, not only Ca^{++} ions may have such a biphasic action on LPO, but also other cations with high charge density, capable of releasing Fe^{++} ions bound with negatively charged groups of lipids, and also of interacting with $O_2^{\cdot}$ radicals [9, 12]. It was found that in the absence of Ca^{++} ions, addition of La^{+++} ions to linolenic acid ufasomes in a concentration corresponding to that of the Fe^{++} ions present stimulated MDA accumulation (Table 1). On the combined action of equimolar concentrations of Ca^{++} and LA^{+++} (when their total concentration exceeded that of Fe^{++}), an effect of inhibition of peroxidation of linolenic acid was observed.

The results as a whole lead to the conclusion that stimulation of LPO by Ca^{++} ions is based on their ability to liberate Fe^{++} ions bound with the negatively charged groups of lipids, thereby increasing the concentration of catalytically active Fe^{++} in the system. Weakening of the stimulating action of Ca^{++} and the inhibitory effect of high concentrations of Ca^{++} ions are due to their interaction with the superoxide anion-radicals. This biphasic character of the action of Ca^{++} on LPO can evidently be realized in LPO induction systems that depend on generation of $O_2^{\cdot}$, and with lipid substrates containing negatively charged groups. In this connection it is worth recalling that a similar biphasic character of action of Ca^{++} on LPO was found previously on natural membranes (microsomal and mitochondrial fractions of myocardium), in which NADH- and NADPH-dependent oxidases functioned at $O_2^{\cdot}$ generating systems [2].

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