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INTERACTIONS OF FERROUS IONS WITH PEROXIDIZED LECITHIN-LIPOSOMES *

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Oxidation of Fe^{2+} in the presence of peroxidized lecithin-liposomes small unilamellar vesicles suspended in various media (pH = 5.5) is described. Peroxidation of lecithin was induced by UV light and assayed by three methods: estimation of conjugated dienes, accumulation of hydroperoxides and by the thiobarbituric acid test. The oxidation od Fe^{2+} was accelerated by phosphate and citrate buffers, but in acetate buffer it was dependent on lipid peroxidases concentration and exibited saturation kinetics.

Lipid peroxidation has been widely investigated because of its possible role in a variety of pathological conditions [1, 2]. Exposure of biomembranes to oxidative stress leads to a progressive degradation of the biomembrane structure resulting in functional changes, such as decrease in membrane fluidity [1, 2], increase in permeability [3], changes in enzymatic activites and transport functions [1]. The role of transition metal ions (iron) in initiating lipid peroxidation has been intensively studied because of its physiological significance [4, 5].

The classical understanding of lipid peroxidation catalyzed by iron [6] suggests the decomposition of lipid peroxides (LOOH) according to the reaction:

$$Fe^{2+} + LOOH \rightarrow Fe^{3+} + LO^{\cdot} + OH^{-}$$

However, recent studies have suggested that an important factor for initiation and propagation of lipid peroxidation by iron in biological membranes is the ratio of Fe^{3+}-chelate:Fe^{2+}-chelate [7, 8, 9], perhaps through the formation of Fe^{3+}-dioxygen-Fe^{2+} [8]. Garnier-Suillerot *et al.* [10] postulated the following scheme for the initial LOOH decomposition by Fe^{2+}, where the first step might involve the binding to phosphatidylcholine (PL)

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forming presumably a 1:1 complex "Fe^{2+}-PL". In the second step the decomposition follows according to:

$$Fe^{2+}\text{-PL} + LOOH \rightarrow Fe^{3+}\text{-PL} + OH^{-} + LO^{\cdot}$$

In the present study, the oxidation of Fe^{2+} under a variety of medium composition and the presence of peroxidized egg lecithin liposomes was examined.

MATERIALS AND METHODS

Multilamellar vesicles (from fresh egg phosphatidylcholine "egg-lecithin", isolated by method of Singleton [11]) were prepared according to the procedure of Bangham [12] using the following buffers (pH 5.5): (1) 0.1 M phosphate, (2) 0.1 M citrate, (3) 0.05 M acetate and (4) distilled water with traces of HCl. Liposome suspensions (lipid concn. 10 mg/ml) were sonicated under N_2 (60 times in 45 s intervals, 4°C) by use of probe-type Ultrasonic Disintegrator, type UD-20, to form small unilamellar vesicles (liposomes).

Oxidation of lecithin in liposomes was carried out by UV light (Lamp UV-VIS, PLK-85, 125 W) irradiation and estimation by the following methods: 1, the diene conjugates by use of the oxidation index [13] expressed as the ratio A233/A215; 2, the lipid hydroperoxides were assayed by the spectrophotometric titration of their reaction product with iodide [14] and expressed as absorbance at 353 nm; 3, the formation of thiobarbituric acid-reactive oxidation products (R-TBA) was determined as described by Buege & Aust [14] with a modification (samples were boiled in the presence of 0.05% butylated hydroxytoluene [8] and were expressed as absorbance at 535 nm.

$Fe(NH_4)_2(SO_4)_2$ solution (600 μM) were prepared in degassed, nitrogen-saturated buffers (1-4) and were added to oxidized liposome suspensions (1:1, v/v) after irradiation. Oxidation of iron was followed after 90 s incubation by measuring the ferrous ion-*o*-phenanthroline complex [10].

RESULTS AND DISCUSSION

Oxidation of lecithin induced by UV irradiation is accompanied by the increase in thiobarbituric-acid oxidation products (R-TBA) (Fig. 1). For up to 8 min of irradiation an asymptotic decrease of the oxidation index value and for up to 4 min a decrease at the concentration of hydroperoxides was observed. One can not exclude that this phenomenon is directly related to oxidation of diunsaturated fatty acids in lecithin. It is shown in Fig. 2 that the oxidation of ferrous ions calculated from the *o*-phenanthroline reaction depends on composition of the medium. In citrate buffer a direct relationship was shown between the buffer and ferrous ion concentration. The development

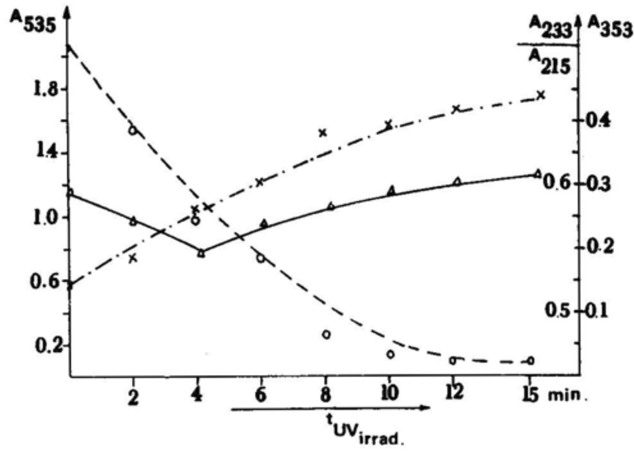

Fig. 1. UV light induced oxidation of lecithin in liposomes SUV suspended in acetate buffer (pH = 5.5). Lecithin concentration: 10 mg/ml. Accumulation of lecithin peroxidation products were determined using: ○, the oxidation index (referred as the ratio A_{233}/A_{215}); △, iodometric assay of hydroperoxides (expressed as absorbance at 353 nm); X, thiobarbituric acid test (expressed as the increase of absorbance at 535 nm)

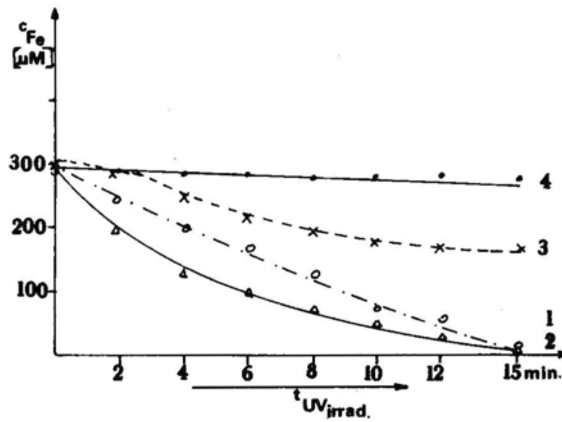

Fig. 2. The oxidation of Fe^{2+} by peroxidized lecithin in liposomal membranes. The oxidation of Fe^{2+} was measured colorimetrically using *o*-phenanthroline method after 90 s incubation (1 : 1; v/v) with peroxidized liposomes SUV in buffers (pH = 5.5): ○, (1) 0.1 M phosphate; △, (2) 0.1 M citrate; X, (3) 0.05 M acetate; ●, (4) distilled water with trace HCl

of R-TBA was observed after a lag-phase (not shown). Similar results were published by Braughler *et al.* [7], where autoxidation of Fe^{2+} was demonstrated to be a reaction with oxygen dissolved in the lipid phase resulting in production of highly reactive hydroxyl radicals. Braughler *et al.* [8] demonstrated that the autoxidation of Fe^{2+} can be involved in production of Fe^{3+}, to form the Fe^{2+}-O_2-Fe^{3+} complex. The report of Minotti & Aust [9] show that citrate buffer is capable of shifting the Fe^{2+}/Fe^{3+} couple reduction potential from -0.77 to -0.33 V, which also increased the rate of Fe^{2+} autoxidation and that the peroxidation of lecithin can occur when the molar ratio Fe^{2+}-citrate:Fe^{3+}-citrate reaches the value of 1:1. Thus, the observed lag-phase of R-TBA can be explained. We have shown, that the decomposition of LOOH groups by Fe^{2+} does not occur during the first 90 s when the liposomes are suspended in distilled water.

In acetate buffer, the oxidation of Fe^{2+} dependend on LOOH concentration and saturating kinetics was observed, similarly as it was shown by Garnier-Suillerot [10], who postulated the formation of Fe^{2+}-PL complexes.

The existence of such Fe^{3+}-PL complexes at the surface of SUV liposomes was investigated by use of IR-spectroscopy [15]. Our IR study also supports the formation of Fe-PL complexes, because differences were shown when Fe^{2+} or Fe^{3+} interacts with oxidized lecithin in liposomal membranes.

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