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Spectroscopic studies of alpha tocopherol interaction with a model liposome and its influence on oxidation dynamics ☆

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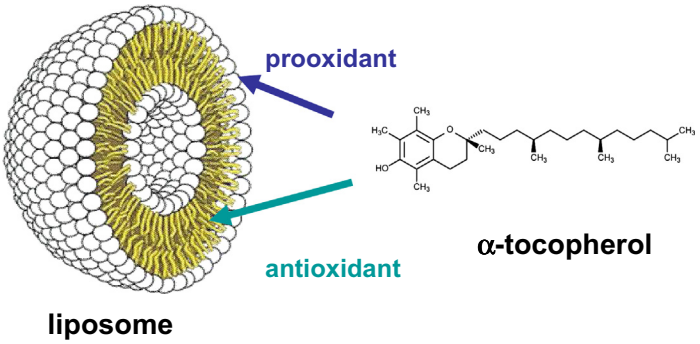
HIGHLIGHTS

- Liposome was used as a model system for lipid monolayer of lipoprotein.
- Interaction of α-tocopherol with liposome was studied by FT-IR spectroscopy.
- Localization of α-tocopherol depended on a way of its import into lipid system.
- Oxidation dynamics of liposome and that with α-tocopherol was studied by EPR.
- Depending on localization, α-tocopherol acts as antioxidant or prooxidant.

GRAPHICAL ABSTRACT



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ABSTRACT

The influence of α-tocopherol on the surface conformation of liposome, as a model component of lipoproteins, and its role in oxidation process were studied. FT-IR spectra from suspensions of neat liposome, mixtures of liposome and α-tocopherol and liposome with incorporated α-tocopherol were analyzed. When α-tocopherol was incorporated into liposome, intensities of some bands were decreased or increased in comparison with the spectra of liposome and α-tocopherol mixture. These changes reflect the different localization of α-tocopherol in two types of liposome suspensions. The oxidation of liposome suspensions was initiated by addition of cupric ions. After prolonged oxidation, the differences in FT-IR spectra of oxidized samples were recorded. Differences were observed in comparison with spectra of native and oxidized liposomes were analyzed. The rate of oxidation was measured by EPR oximetry. Oxidation was generally very slow, but faster in liposome without α-tocopherol, indicating the protective role of α-tocopherol against liposome oxidation. On the other hand, liposome suspensions with EDTA in the buffer were not oxidized at all, while those with α-tocopherol and liposome mixture were only slightly oxidized. In this case the consumption of oxygen was the result of liposome oxidation supported by α-tocopherol. These results reflect the ambivalent role of α-tocopherol in liposome oxidation, similarly to findings in studies of lipoprotein oxidation.

Introduction

Lipoprotein oxidation, mainly of low density lipoprotein (LDL), and its influence on atherogenesis was extensively studied *in vitro* from the basic chemistry to the biological effects of oxidized LDL on culture cells [1]. *In vivo* clinical studies were

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mostly focused on understanding the role of antioxidants, specifically α-tocopherol, in prevention of the development of atherosclerosis. Earlier studies, where strong oxidation of LDL initiated by transition metals was performed *in vitro*, confirmed the protective role of α-tocopherol acting as a trap for peroxyl radicals formed in oxidation chain. If the oxidation hypothesis of atherogenesis were to be correct, it was expected that the regular uptake of vitamin E supplements would prevent or slow down the progress of atherosclerosis. However, numerous clinical studies failed to confirm that the uptake of α-tocopherol had any influence on the development of atherosclerosis and cardiovascular disease [2].

Later studies of LDL oxidation *in vitro* demonstrated that under some conditions, α-tocopherol could act as prooxidative agent, increasing the rate of oxidation. This process was called tocopherol mediated peroxidation (TMP) and suggested at first by Stocker and his group [3]. Our group had investigated the slow oxidation of LDL in a closed system by EPR measurement of oxygen concentration using spin probe [4]. On the basis of these experiments we had built the theoretical probabilistic model of LDL oxidation in which α-tocopherol in interaction with transition metal was acting as a prooxidant on one site of the oxidation cycle and as an antioxidant on the other site [5].

Results from literature confirmed that oxidation process is not limited only to the lipid domain of the particle but free radicals were transferred to the protein domain, by reaction of phospholipids with lysine groups [6]. EPR spin trapping experiments of lipid and protein fractions of oxidized LDL also demonstrated that larger number of radicals was trapped in protein domain [7]. Fluorescent measurements of LDL oxidation demonstrated that it starts by decomposition of tryptophan residues in apolipoprotein B, immediately after addition of cupric ions, with subsequent reaction of tryptophan peroxides with lipids [8]. This process did not depend on α-tocopherol.

In order to get more insight into interaction of α-tocopherol and the lipid domain of lipoprotein, in our experiment liposome was used as a model of LDL particle. Three types of liposome suspensions were prepared: neat liposome in suspension with EDTA and without EDTA, liposome suspension with added α-tocopherol to preformed vesicles, and liposome suspension into which α-tocopherol was added to the mix of lipids and cholesterol before the formation of vesicles.

We applied FT-IR spectroscopy to inspect the possible conformational differences in the spectra of liposome induced by the proximity or incorporation of α-tocopherol into vesicles.

The oxidation was initiated by addition of small amounts of $CuCl_2$ to the suspension. The oxidation was followed by measuring oxygen consumption in aqueous domain using EPR oximetry [4].

FT-IR spectra of oxidized liposome samples were recorded and compared with the spectra native samples. Differences in spectra indicated the conformation changes due to oxidation.

Experimental

All chemicals were purchased from Sigma Aldrich and used without further purification.

Preparation of liposome vesicles

Stock solutions of phosphatidylcholine (PC), 20 mg/2 mL, sphingomyelin (SM), 15 mg/1 mL and cholesterol (C), 20 mg/2 mL were prepared in chloroform–methanol (2:1, v/v). The aliquots were combined to obtain the molar ratio of PC:SM:C as 2:1:0.3. The solvent was slowly removed from lipid solution under a stream of nitrogen and additionally under vacuum for 1 h. The lipid film was further dispersed in 2 mL of 0.1 M phosphate buffer saline (PBS) by shaking for 5 min in vortex. For some oxidation experiments, EDTA was added to the buffer solution. EDTA is widely used in biological systems as a chelator for the metal ions and therefore is considered as a protector against the oxidation. The final concentration of liposome in the buffer was 1.65 mmol/mL. The sonication of the liposomal solution led to formation of unilamellar vesicles. The formation of vesicles was tested in the images obtained by transmission electron microscope using facility at Ruđer Bošković Institute in Zagreb.

α-Tocopherol solutions

α-Tocopherol was dissolved in chloroform–methanol (2:1, v/v), 20 mg in 2 mL as a stock solution. The aliquots of tocopherol solution were added to liposome suspension in a ratio of one molecule of α-tocopherol to 7 molecules of lipids for IR measurements and one molecule of α-tocopherol to 56 molecules of lipids for EPR measurements. In the other experiment 0.66 mmol of α-tocopherol was added to lipid and cholesterol mixture to obtain vesicles with 20% embedded α-tocopherol.

Copper(II) chloride dihydrate solutions

Cupric ions were used as the initiators of lipid peroxidation. Stock solution of $CuCl_2{\cdot}2H_2O$ was prepared by dissolving 20.46 mg $CuCl_2{\cdot}2H_2O$ in 10 mL of PBS to obtain the concentration of 12 mmol/mL. This stock solution was further diluted to obtain the desired concentration in aliquots of 10 μL which were added to the liposome suspensions. The ratios of cupric ions to lipids in various samples were 1:10, 1:20, 1:50 and 1:100.

Oxidation measurements

Spin probe 3-carbamoyl-2,2,5,5-tetramethyl-3-pyroline-1-yloxy (CTPO) was dissolved in PBS, 4.18 mg/100 mL. The concentration of solution was 0.22 mM. The final concentration of CTPO in the sample was 0.11 mM. Copper and CTPO solutions were added to the liposome suspension immediately before incubation. Samples were incubated in sealed quartz micropipettes at room temperature for two days before EPR measurements.

EPR spectrum of CTPO consists of triplet of nitrogen lines with super hyperfine pattern of 10 lines from 4 methyl groups. Their resolution is sensitive to the concentration of oxygen in the aqueous phase of the sample. Oxygen concentration was determined from EPR spectral parameters of CTPO, using calibrated curves from the literature [9].

FT-IR spectroscopy

For the spectroscopic measurements, the liposome solution (10 μL) was placed on ZnSe window and dried under nitrogen for 20 min to obtain thin film. The film was further dried under vacuum for another 15 min.

The oxidized samples were obtained by prolonged incubation of liposome suspensions with added cupric ions (1 per 10 lipids). FT-IR spectra were recorded with PerkinElmer Spectrum GX spectrometer equipped with a DTGS detector. Each spectrum was obtained from 100 co-added scans in the region 4000–600 cm^{-1}, with 4 cm^{-1} resolution, at room temperature. The spectrometer was continuously purged with nitrogen to remove the water vapor from the detector and the sample compartment.