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Photostability of alpha-tocopherol ester derivatives in solutions and liposomes. Spectroscopic and LC–MS studies

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ABSTRACT

α-Tocopherol (Toc) is known to degrade to the tocopheroxyl radicals (Toc•) by exposure to UV light irradiation. In the present study, the stability of Toc ester derivatives exposed to UV light was investigated and compared with Toc in organic solution and in phospholipid vesicles. To follow the depletion of Toc and its esters the absorbance and fluorescence methods were applied whereas degradation products were detected using LC–MS method.

The irradiation with UVB light of air-equilibrated solutions of di-α-Tocopheryl malonate (DTMO), α-Tocopheryl malonate (TMO) and α-Tocopheryl succinate (TS) strongly modifies their absorption and fluorescence spectra. Upon UVB irradiation, absorption band at 279/285 nm becomes less pronounced indicating the photodegradation of esters. During irradiation, the fluorescence maximum of esters at 305 nm shifts to 326 nm, a maximum characteristic for Toc. Photorecovery of Toc from its esters derivatives was finally confirmed by LC–MS method. Among studied esters, only α-tocopheryl nicotinate (TN) did not undergo depletion and appeared resistant to UVB radiation. Kinetic studies indicated that photoinduced transformation occurs through the first order consecutive reaction chain mechanism. The photodissociation of Toc esters in the liposomes occurred with one order of magnitude slower than in organic solvents. Using MS/MS method it was found that final stable product of irradiation was α-tocopheryl quinone (TQ), an animal and plant metabolite of Toc.

1. Introduction

α-Tocopherol (Toc), except its antioxidant function, fulfills a number of biochemical and biological functions in the cell. However, Toc is unstable under the influences of light and air. It has been shown that Toc under UV light irradiation is easily decomposed to the tocopheroxyl radicals (Toc•) [1,2]. Several studies have shown that photooxidation processes lead to loss of antioxidant activity of vitamin E [3–5]. Such photochemical generation of free Toc• by UV light is often a large problem for biological systems containing vitamin E because Toc• is supposed to initiate and accelerate the lipid peroxidation (prooxidation) [6].

Reported studies have shown that during irradiation of Toc depending on the range of light several products may be formed. Irradiation of Toc in methanol and hexane under 366 nm leads to its degradation with first order kinetics [7]. Such process was attributed to the formation of methoxy radicals, hydrogen peroxide formation and conversion of Toc to phenoxy radicals. Another research has shown that during irradiation of tocopherol in acetonitrile the formation of Toc• and its cationic form were observed whereas in methanol only the formation of Toc• occurred [8]. UVB (280–320 nm) irradiation of thin films of free Toc on Petri dish yielded a mixture of dihydroxydimer, spirodimer, and spirotrimer [9]. Irradiation under sunlight and UV light ($\lambda > 290$ nm) of Toc in olive oil and hexane leads to its depletion with a few products and two of them were identified by HPLC as 5-formyl-γ-tocopherol and α-tocopheryl quinone (TQ) [10]. Irradiation with UVB of γ-Toc in acetonitrile/H_2O solution and in soy phosphatidylcholine (PC) and dipalmitoyl phosphatidylcholine (DPPC) liposomes leads to formation of photoproducts which include TQ, epoxyquinones, hydroperoxy-tocopherones, Toc• and spirodimers [11]. TQ and its reduced form, α-tocopheryl hydroquinone (α-TQH2), are found among oxidation products of Toc in both animal and plant metabolisms [12,13].

Commercially, vitamin E supplements contain mostly esterified forms e.g. acetate, succinate or nicotinate. Toc succinate and acetate do not function as antioxidants, but can serve as a source of a free Toc upon deesterification by the proper enzymes. Vitamin E and its esterified forms can be exposed to sunlight or artificial UV light in several processes like skin under sunlight [14,15], preparation or refinement of foods [16] or in many processes in pharmaceutical or cosmetic industry [17,18]. So, the study on irradiation process of the commercial forms of vitamin E may provide some knowledge about the role of exposure to light influences the stability of the esterified form of vitamin E.

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As mentioned above, several studies of photodegradation processes of Toc were presented in the literature, but more detailed studies devoted to UV irradiation of tocopherol esters are rather scarce. Published studies were mostly focused on irradiation of topically applied esters, acetate or succinate, and its protection effect in photocarcinogenesis caused by UV irradiation [14,17,19]. The results showed that the esters are not cleaved when applied to the dermis and epidermis. The process of bioconversion to free Toc when the acetate was topically applied and irradiated with UVB was reported [19]. However, according to the author's interpretation this process was not connected with irradiation. The photostability of Toc acetate was studied by treating ethanol solution with 254 nm or 366 nm light [20]. Decrease of native fluorescence was observed during UVC (200–280 nm) irradiation whereas applied UVA (320–400 nm) did not lead to degradation of Toc acetate.

To determine the photostability of Toc derivatives, we investigated the influence of UV light on the physicochemical properties of two novel esters: di-α-Tocopheryl malonate (DTMO) and α-Tocopheryl malonate (TMO), and synthesized α-Tocopheryl succinate (TS) as well as α-tocopheryl nicotinate (TN) (Fig. 1). The effects of irradiation of Toc esters were observed in organic solvents as well as in lipid membranes and compared with native Toc. To follow the fate of Toc and its esters the absorbance and fluorescence methods were applied whereas detection of the degradation products was performed with LC–MS method.

2. Materials and Methods

2.1. Materials

Egg yolk phosphatidylcholine (PC), dipalmitoyl phosphatidylcholine (DPPC), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), chloroform and methanol were purchased from Sigma/Merck (Germany). Toc, TMO, DTMO, TN and TS were synthesized according to procedures presented in [21,22]. Ethanol, hexane, n-octane and acetonitrile were purchased from POCh (Poland). All solvents were of spectroscopic grade. The ultra pure water from MicroPure Water System manufactured by TKA (Germany) was used in all aqueous solutions.

2.2. Methods

2.2.1. Preparation of Vesicles

Large unilamellar vesicles (LUV) with the incorporated Toc esters were prepared by the extrusion method. The details of this procedure are given elsewhere [23]. Shortly, Toc and its esters were dissolved in methanol to give a stock solution. For liposomes preparation of dry DPPC or PC and esters were dissolved in chloroform and methanol, respectively, mixed in different proportions and then the solvent was evaporated. The formed film was hydrated with 0.1 M phosphate buffer (pH 7.4) and vortexed for 10 min. The resulting liposomal suspension (0.08 mg/ml phospholipid final concentration) was extruded using a LiposoFast Basic LF-1 extruder (Avestin Europe GmbH, Mannheim, Germany) with 100 nm pore diameter.

The mean particle diameters of the LUV suspension were measured using the method of dynamic light scattering with Zetasizer Nano (Malvern Instruments, Worcestershire, UK) [24]. The mean values of DPPC and PC liposome sizes: 120 and 105 nm, respectively, were determined from the liposomal size distribution histograms and were not changed significantly in the present of studied compounds.

2.2.2. UVB Irradiation

The sample was irradiated with a Xenon lamp (50 W). Irradiation treatment was carried out at either 290 nm for Toc and at 280 nm for the esters (UVB) or at 320 nm for all studied compounds (UVA). The intensity used in the experiment was 14 and 20 mW/cm^2 of UVB and UVA, respectively, as measured by a research radiometer (LM2, Carl Zeiss Jena, Germany). During irradiation, the solution was gently mixed with a magnetic stirrer. All samples were kept at 25 °C during exposure.

After various times of irradiation indicated in the text the cuvette was removed from the UV beam to measure absorption and emission spectra. The sample was then brought back to the irradiator, and the cycle was repeated until the spectrum of the sample did not change noticeably between two successive irradiations.

2.2.3. Absorbance, Fluorescence and Fluorescence Lifetime Measurements

Absorption spectra were measured with a Shimadzu UV 1202 spectrophotometer (Shimadzu, Kyoto, Japan) with a spectral resolution of 1 nm. Steady-state fluorescence emission spectra were recorded on a Shimadzu RF 5001PC spectrofluorometer (Shimadzu, Kyoto, Japan) with a spectral resolution of 1 nm. Fluorescence lifetime measurements were carried out with a TimeHarp 100 PC-board (PicoQuant, Berlin, Germany) for time-correlated single photon counting (TCSPC) with 72 ps/channel resolution. The excitation source was a sub-nanosecond pulsed UV LED 290 with maximum emission centered at 290 nm and a 0.7 ns wide pulse with full width at half maximum (FWHM), powered by a PDL 800-D driver. The emission was detected by a PMA 182 photon sensor head (all the instruments from PicoQuant, Berlin, Germany). The data were analyzed by an exponential reconvolution method using a non-linear least square fitting program. Optimum fitting with minimisation of the residuals was confirmed using a Chi-squared value $\chi^2 < 1.4$. All spectroscopic measurements were performed using a 1 or 0.3 cm path length quartz cuvette at 22 °C.

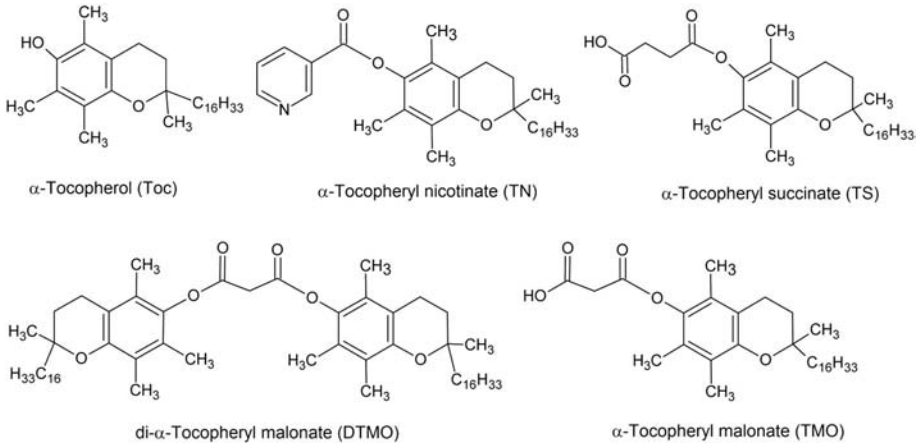

Fig. 1. Chemical structures of studied compounds.