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FORMATION OF COMPLEXES OF α-TOCOPHEROL WITH PHOSPHATIDIC ACID

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Activation of phospholipases in the animal cell is an essential step, under certain conditions, in the course of several metabolic and functional processes [1]. In particular, phospholipase D takes part in the synthesis of ceramines – an important component of brain lipids [8, 5]. Meanwhile activation of phospholipases means not only guaranteeing the course of essential processes, but also modification of some of the physicochemical parameters of biological membranes, including brain synaptic membranes [5]. This state of affairs explains why protective mechanisms against the harmful action of phospholipases are essential in order to maintain cellular homeostasis.

It has recently been shown that α-tocopherol stabilizes synaptosomal membranes against the harmful action of phospholipase A_2 [5]. This stabilizing effect is due to the ability of α-tocopherol to form complexes with free fatty acids (FFA) [4, 7]. Investigation of the nature of complexes of α-tocopherol with FFA has shown that this process takes place through two types of interaction: polar interaction between the OH-group of the chromane ring of α-tocopherol and the carboxyl group of the fatty acid and hydrophobic interaction of cis-unsaturated double bonds of the fatty acid with methyl groups of the chromane ring of α-tocopherol [2].

Since complexes of this kind can potentially be formed with a fairly wide class of compounds [3, 6], the possibility of formation of complexes of α-tocopherol with phosphatidic acid (PA), the principal hydrolysis product of phospholipids by phospholipase D, was investigated.

EXPERIMENTAL METHOD

α-Tocopherol was obtained from Serva (West Germany), PA was obtained by phospholipid hydrolysis of ovolecithin (PAL), and dimyristoylphosphatidic acid (DMPA) were from Koch-Light (England), and deuterated chloroform from Merck (West Germany). Heptane was purified by redistillation.

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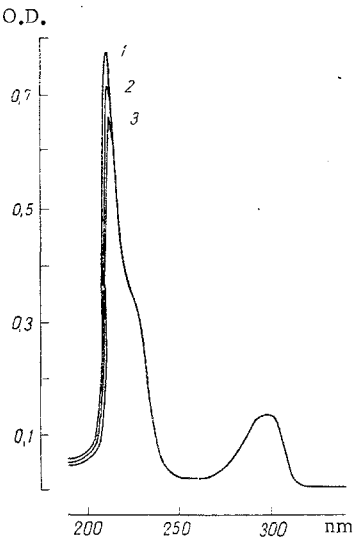

Fig. 1. UV absorption spectra of solution of α-tocopherol in heptane (10^{-5} M) at room temperature before (1) and after addition of PAL to the cuvette (2, 3). Concentration of PAL was 0.36 mg/ml (2) and 0.72 mg/ml (3). The same quantity of PAL was added to the comparison cuvette.

UV absorption spectra were measured on a 220A spectrophotomer (Hitachi, Japan), and ^{1}H-NMR spectra were recorded on a WH-400 spectrometer (Bruker, West Germany). The residual signal of chloroform (δ = 7.25 ppm) was used as internal standard. The results were subjected to statistical analysis on the CX-1 minicomputer (Canon, Japan).

EXPERIMENTAL RESULTS

It was shown previously that the formation of complexes of α-tocopherol with FFA is accompanied by a change in the absorption of α-tocopherol at 206-210 nm [4, 7]. UV spectra of solutions of α-tocopherol in heptane before and after addition of increasing quantities of PAL are illustrated in Fig. 1. They show that addition of PAL to the solution caused a decrease of optical density in the 205-209 nm region but did not change absorption at the maximum at 293 nm. Similar changes were observed in the spectrum after addition of DMPA to the solution. Changes in optical density were observed virtually instantaneously and were proportional to the concentration of added PA. Considering this fact, equilibrium constants of interaction of α-tocopherol with PA could be calculated as described previously [4, 7]:

$$K = \frac{A_0 - A}{A_0 \cdot [PA]},$$

where A_0 and A represent optical densities at 206 nm before and after addition of PA respectively. Constants of interaction of α-tocopherol with PAL and DMPA, calculated in this way, were $(1.4 \pm 0.1) \cdot 10^3$ and 76.6 ± 13.2 M^{-1} respectively. The considerable difference in constants of interaction of α-tocopherol with DMPA and PAL indicates that in the case of interaction with FFA the presence of double bonds in the lipid plays an essential role in formation of the complex and determines the magnitude of the constant of interaction.

By high-resolution ^{1}H-NMR spectroscopy it is possible to identify groups of atoms taking part in formation of the complex of α-tocopherol with PA. The ^{1}H-NMR spectrum of α-tocopherol in deuterated chloroform is shown in Fig. 2. In the spectrum the signal with δ = 4.31 ppm corresponds to protons of methyl groups of the chromane right, the doublet with δ = 2.10-2.15 ppm corresponds to protons of methyl groups of the chromane ring the signal with δ = 1.21 ppm corresponds to protons of methylene groups of the phytol chain, and the multiplet in the region 0.82 ppm corresponds to protons of methyl groups of the phytol chain.

On addition of PAL to the α-tocopherol solution considerable widening of the signal of the OH-group and widening of the signal of the methyl groups of the chromane ring were observed. The remaining groups of signals in the NMR spectrum of α-tocopherol were not widened. Similar widening of signals of the OH-group

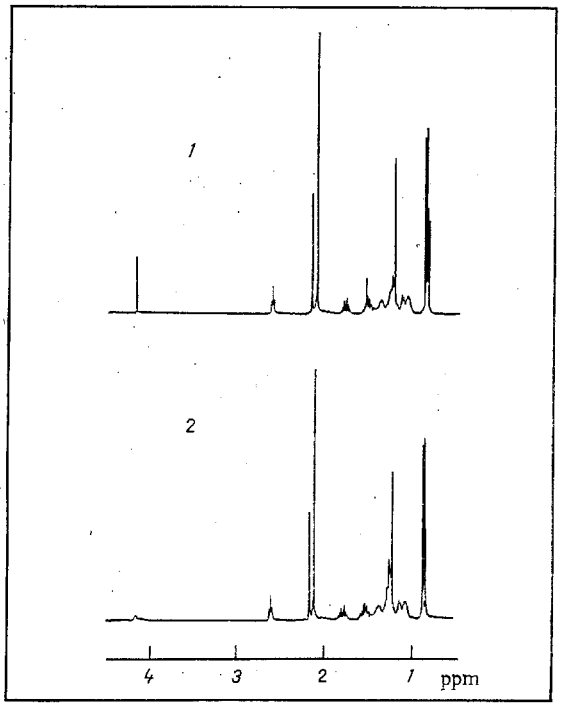

Fig. 2. ^{1}H-NMR spectrum of α-tocopherol (1) and of mixture of α-tocopherol with PAL in the ratio of 5 : 1 (2) in deuterated chloroform at 300°K.

and methyl groups of the chromane ring of α-tocopherol was observed previously during interaction of α-tocopherol with FFA [3]. These results, as well as those obtained previously [3], justify the conclusing that during interaction of PAL with α-tocopherol a complex also is formed on account of two types of interaction, namely: interaction of the OH-group of the chromane ring of α-tocopherol with the polar group of PAL and hydrophobic interaction of cis-unsaturated double bonds of acyl residues of PAL with methyl groups of the chromane ring of α-tocopherol.

Incidentally, the constant of formation of the PA– α-tocopherol complex is significantly increased by an increase in the degree of unsaturation of the acyl residues of PA. During hydrolysis of phospholipids by phospholipases, including by phospholipase D, unsaturated phospholipids are the first to undergo conversion [1]. It is thus clear why α-tocopherol in biological membranes will interact first with unsaturated PA, and not with more saturated phospholipids. Taking this into consideration, and also the high values of constants of interaction with PA with α-tocopherol in the solution, it can be tentatively suggested that interaction of α-tocopherol with PA in biological membranes will take place with quite high intensity. This, in turn, indicates that the formation of complexes of α-tocopherol with PA in biological membranes and, in particular, in synaptic membranes, can be regarded as a possible molecular mechanism of the stabilizing action against injury caused by phospholipase D.

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