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Partition of tocopheryl glucopyranoside into liposome membranes studied by fluorescence methods

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ABSTRACT

Vitamin E is poorly soluble in aqueous solutions. Enhanced physiological activity is expected from synthesized glycosidic tocopherol derivatives. We investigated binding, location and interactions of newly synthesized DL-α-tocopheryl β D glucopyranoside (II) in phosphatidylcholine liposomes using fluorescence emission, anisotropy and lifetime methods. In liposomes emission maximum and fluorescence lifetime of glucoside were similar to those observed in methanol. High fluorescence anisotropy value indicates that tocopheryl glucoside is located in restricted mobility region of the membrane. Thermodynamic calculation indicated efficient partition of (II) into membrane. The energy minimization calculations of electrostatic potential distribution of (II) and solvation energies performed with Gaussian program confirmed strong affinity of glucosidic moiety for ionic interactions and supported proposed model of interactions. The all obtained data indicate that DL-α-tocopheryl β-glucoside is embedded into the membrane interior whereas sugar moiety protrudes above the water/lipid interface of the membrane surface.

1. Introduction

Vitamin E is known for its strong affinity to biomembranes through interactions with membrane phospholipids [1–4]. However, due to its poor absorbability and bioavailability its clinical application is limited. Enhanced physiological activity is expected from synthesized tocopherol derivatives, whose physiological activity of vitamin E is enabled by the enzymatic hydrolysis of the glycoside bond. The reported attempts to obtain readily hydrolysable derivatives include the synthesis of various tocopherol glycosides [5–10] by introducing a sugar group at C-6 position of the chromanol ring. Contrary to the expectations, some tocopheryl glycosides prove resistant to hydrolysis; however, some other types of physiological activity were observed in these compounds [5,11]. Glucosides and mannosides proved to suppress histamine release; inhibition of IgE antibody production was observed, too [8]. The reported effects of tocopherol glycosylation open the door to new medicinal applications. Physicochemical and spectroscopic studies allow to get an insight into the location of these tocopherol derivatives in biological membranes and their expected interactions with membrane components. In this study we use intrinsic fluorescence properties of the investigated derivatives of DL-α-tocopherol for direct observation of their behavior in membranes (Fig. 3).

The primary aim of this study is to investigate the nature of the interactions and the location of the studied glucoside in a biomembrane model system. This is done through investigating photophysical properties (absorption and fluorescence spectra, fluorescence anisotropy and fluorescence lifetime) quantum-chemical calculation of solvation energies of DL-α-tocopheryl β-glucoside in phospholipid model membranes through correlating the spectroscopic properties of the glycoside and the physicochemical properties of the solvents. In this work, mechanisms and type of interactions between newly synthesized tocopheryl glucoside and PC and DPPC membranes were investigated systematically by spectroscopic and quantum-chemical methods for the first time.

2. Materials and methods

2.1. Materials

DL-α-tocopherol (**I**) was purchased from SIGMA. DL-α-tocopheryl β-D-glucoside (**II**) (Fig. 1) was synthesized according to Witkowski and Walejko [12]. Used organic solvents of spectral grade purity were acquired from Merck (Germany). The water used came from a MicroPure Water System manufactured by TKA (Germany).

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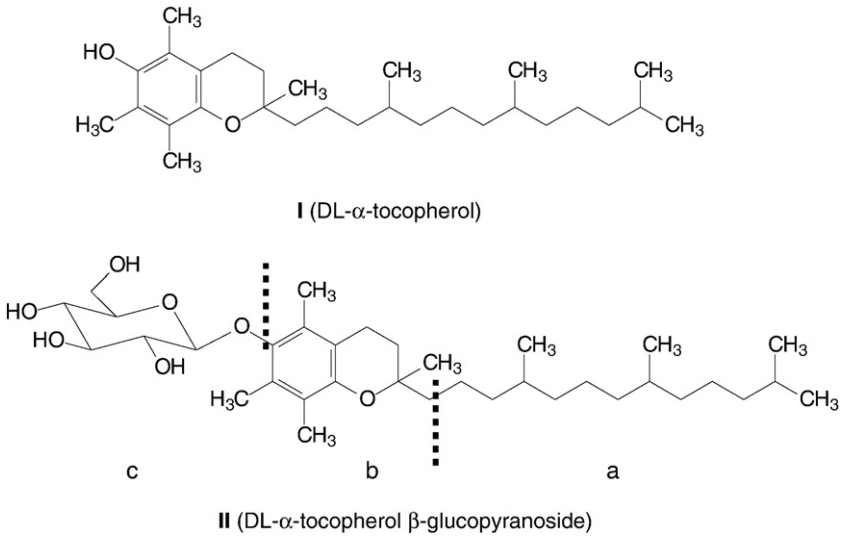

Fig. 1. Chemical structures of α-tocopherol (**I**) and its glucosidic derivative DL-α-tocopheryl β-glucoside (**II**). The broken lines and indexes a, b, c define the functional groups of (**II**) for which the solvation energy was calculated separately.

2.2. Liposome preparation

Egg yolk phosphatidylcholine (PC) and dipalmitoyl phosphatidylcholine (DPPC) were purchased from SIGMA. The liposomes with the cosolubilized glycosidic derivatives were prepared by the extrusion method. For liposomes preparation dry DPPC or PC was dissolved in hexane, glycosides were dissolved in methanol then both solutions were 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 at 50 °C (for DPPC) or 22 °C (for PC) respectively, until a clear solution was obtained and cooled down to 22 °C (for DPPC). The resultant liposomal suspension was extruded using LiposoFast Basic LF-1 extruder with 100 nm diameter membrane. Double distilled, deionized water was used.

2.3. Spectroscopic measurements

Steady-state fluorescence spectra were taken with a Shimadzu RF 5001PC fluorometer, and absorption spectra with a Shimadzu UV 1202 spectrophotometer. Fluorescence lifetime measurements were carried out with a PicoQuant TimeHarp 100 PC-board for time-correlated single photon counting 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, Germany). A 300 nm interference filter (Zeiss, Germany) was used as excitation window to avoid spectral leaking from the LED. A 325 nm interference filter was placed in front of the detector on the emission side. The data were analyzed by an exponential reconvolution method using a non-linear least square fitting program. The time-resolved data in solvents and liposomes were best fitted with a single exponential decay function. However, a biexponential decay function was also applied and in both cases the goodness of fit was estimated by using χ^2 values.

All the spectroscopic measurements were performed in 1×1 cm quartz cuvette; concentrated solutions were measured in 0.3×0.3 cm cuvette at 22 °C, unless another temperature value is reported in the text.

2.4. Fluorescence steady-state anisotropy measurements

Fluorescence anisotropy measurements were performed using Perkin-Elmer LS-55 fluorometer at 22 °C using appropriate instrument procedure for anisotropy calculation. An excitation wavelength at 280 nm with bandwidth of 10 nm was used. The emission was detected at 315 nm with 10 nm bandwidth. The contribution of scattered light to measured anisotropy was determined independently for reference solution without glycosides. The reported anisotropy values were averaged from several measurements.

2.5. Quantum-chemical calculations

The energy of tocopherol-glucoside was minimized at the B3LYP/6-31G(d) level of theory followed by single point and density calculations at B3LYP/6-311++G(2d,2p) levels. Solvation free energies in 298 K and 1 atm were calculated at the B3LYP/6-31G(d) level. Solvent effects were included using SMD continuum solvation model [13]. The molecular electrostatic potential mapped onto the isodensity surfaces of 0.01 e/Bohr was calculated. Calculated free energies for (I), (II) and functional groups of (II) in water, DMSO and n-pentadecane are presented in Table 2. Calculations were carried out using the Gaussian 09 program [14], and the electrostatic potential was visualized using the Mollso program [15].

3. Results

3.1. DL-α-tocopheryl β-D-glucoside (II) in liposomes

The spectroscopic data obtained for DL-α-Toc (**I**) and DL-α-tocopheryl β-glucoside (**II**) in PC and DPPC liposomes, along with the data obtained in methanol and DMSO/water, are specified in Table 1. It shows similar position of the lowest absorption maximum of (**II**) in all investigated media. Together with the similar molar extinction coefficients the obtained results indicate that in all environments the measured absorption arises from this same, neutral form of glucoside (**II**).

In both liposomes the emission maximum is located at 315 nm compared to 325 nm observed in aqueous DMSO solution and at 313.5 nm in methanol. The obtained differences in Stokes shifts between aqueous solution and liposomes indicate the interactions of glucoside with PC and DPPC membranes. The observed increasing intensity of glucoside fluorescence on addition to liposomes confirms its incorporation in the membranes, inset in Fig. 2.

Fluorescence intensity decay of glucoside in both membranes and in solvents was best fitted with single exponential decay, and data are listed in Table 1. The lifetime of glucoside (**II**) measured in PC and DPPC membranes is similar to those observed in methanol and DMSO/water

Table 1
Positions of absorption and emission maxima and fluorescence lifetimes of 50 μM DL-α-tocopheryl β-glucoside (**II**) and DL-α-tocopherol (**I**) in liposomes, methanol and DMSO/H_2O (92%:8%) solution.

	Absorption, nm	Emission, nm	Lifetime, ns
DL-α-tocopheryl glucoside			
Methanol	287	313.5	3.3
DMSO/H_2O	288	325	3.6
DPPC	289	315	3.3
PC	289	315	3.6
DL-α-tocopherol			
Methanol	295	325	1.5
DMSO/H_2O	294	328	1.45
DPPC	296	325	1.15
PC	296	325	1.2

Calculated from deconvolution of two instrumental response functions, the estimated value of lifetime resolution is $\Delta\tau = 0.05$ ns. The fluorescence data were taken at 22 °C, with excitation wavelength of 290 nm.

solution. The monoexponential fluorescence decay suggests that glucoside is equally distributed in the investigated membranes. For the sake of comparison we also measured in both membranes the fluorescence lifetimes of tocopherol, DL-α-Toc (**I**), Table 1, and the obtained values are similar to those reported in the literature [16,17]. The data collected in Table 1 suggest that the presence of glycosydic moiety at chromanol ring is the main factor which increases lifetime whereas the microenvironment seems to be of lower importance.

The steady-state and time resolved fluorescence emission data suggest that glucosidic molecules are uniformly distributed in PC and DPPC liposomes at similar sites. Whereas both liposomes differ in hydrophobic core and their polar-head group is this same. Thus we may expect that in both liposomes the tocopheryl glucoside molecule is located in this same hydrophilic region of the membrane. The stabilization in the polar-head membrane region is presumably achieved by the electrostatic interactions with positively charged nitrogen center of the choline.

Our earlier studies on the absorption and emission data and the fluorescence lifetimes of glucoside (**II**) in homogenous solutions provided a basis for deducing the location of the studied compound in PC and DPPC liposome model membranes [18]. Both, the fluorescence lifetimes and the spectra of (**II**) in the investigated membranes are similar to those obtained in methanol, which suggests that the glucoside molecule is uniformly distributed in medium value dielectric constant, low viscosity membrane microenvironment.

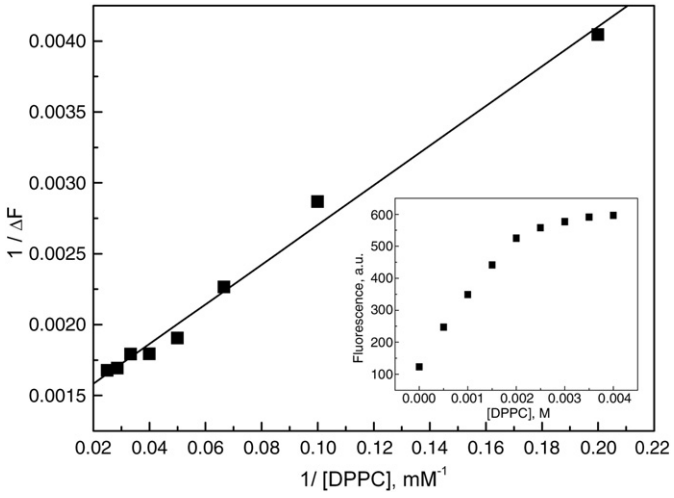

Fig. 2. Double reciprocal plot drawn for the calculation of the partition constant K_b characterizing the partition of α-Toc β-glucoside (**II**) into DPPC membrane. The inset shows the original data of fluorescence intensity with increasing concentration of lipid.

3.2. Partition studies in PC and DPPC membranes

The emission intensity enhancement with saturation trend was found in both liposomes with increasing membrane concentration, inset in Fig. 2. This suggests the interactions between the glucoside and the membrane. The most fluorescence arises from glucoside located in the membrane since in water this molecule is practically nonfluorescent and its concentration is negligible. Thus inclusion of glucoside into membrane interior may be considered as association of glucosides to n binding sites of equal probability. For a quantitative estimation of the interactions the partition constant K_b, was determined from the fluorescence intensity changes on the basis of Eq. (1) [19–22]. This equation was used in a method described by Huang and Haugland [19].

$$\frac{1}{\Delta F} = \frac{1}{\Delta F_{max}} + \frac{55.6}{K_b[M]\Delta F_{max}} \quad (1)$$

where $\Delta F = F - F_0$ and $\Delta F_{max} = F_s - F_0$; F, F_0 and F_s are the fluorescence intensities of glucoside (**II**) in the presence of membrane, without membrane and the membrane concentration at saturation, respectively; K_b is the partition constant; 55.6 and $[M]$ denote the water and lipids concentration, respectively.

The measurement results in DPPC presented as a double reciprocal plot were shown in Fig. 2. The obtained good linearity, as predicted by Eq. (1), confirms the presence of interaction between the studied glucoside and PC and DPPC membrane. The values of the partition constant K_b calculated from the plot for PC and DPPC are 64.2×10^3 M^{-1} and 55.2×10^3 M^{-1}, respectively. The obtained values were found to be of the same order of magnitude to that reported for the other ligands partitioned into membranes [19–22]. The lower value obtained for DPPC membrane reflects the fact that at measurement temperature the DPPC membrane exists in gel phase with the tight lipid packing compared to PC. The partition coefficient values were found to be of the same order of magnitude as reported for tocopherol in model membranes [23,24]. This suggests that the presence of glucoside moiety does not significantly influence the interactions between the membrane and tocopherol as a part of the glucoside molecule. The obtained partition coefficient allowed to calculate standard free energy change ($\Delta G^0 = -RT \ln K_b$) and yields the ΔG^0 value of −27.1 kJ/mol and of −26.7 kJ/mol at 295 K, for PC and DPPC, respectively. The negative values indicate spontaneous partition of the glucoside (**II**) into PC and DPPC membranes.

3.3. Fluorescence anisotropy

Fluorescence anisotropy is used as a sensitive indicator of motional freedom of the fluorophore. The steady-state fluorescence anisotropy increased fast with saturation tendency with rising glucoside content in both membranes. Anisotropy increases from 0.05, observed in solvents, to the highest value of 0.262 obtained at 3 mol% concentration. This anisotropy value remains constant up to 16 mol% of glucoside concentration. To estimate how the presence of glycosidic moiety may influence the mobility of the whole molecule we measured anisotropy for DL-α-Toc in both membranes and the obtained values were equal to 0.23. Similar anisotropy value of 0.22 at 3 mol% concentration of α-Tocopherol in PC membrane was reported [25]. These results suggest that the presence of the glucoside moiety attached to tocopherol molecule does not change significantly its mobility in both membranes. Similar anisotropy value obtained for glucoside (**II**) in PC and DPPC membranes suggests that the tocopherol part resides in confined part of the membrane with restricted motional freedom.

The anisotropy values reported for α-Tocopherol (**I**) in PC and DPPC membranes were close to its reported maximal anisotropy value [25], which is an indication that the tocopherol molecule resides in motionally restricted area of the membrane. At temperature at which the measurements were performed the two membranes are in different morphological phases. PC was in liquid crystalline state

Table 2
Calculated solvation free energies ΔG^0 [kcal/mol] for (I), (II) and functional groups of (II).

	Water	DMSO	n-Pentadecane
I	−0.40	−8.05	−15.57
II	−13.27	−18.85	−16.47
a	2.45	−2.82	−6.02
b	−0.38	−5.15	−7.10
c	−22.87	−15.19	−8.26

Details of calculations are described in Section 2.4.

whereas DPPC was in gel phase; however, emission and lifetime data obtained in both membranes were similar. Similar results of equal rotational freedom for α-Toc in both types of membranes were also reported [25]. This phenomenon may be rationalized by the fact that the hydrophilic part in both membranes is the same with some variation in viscosity. The viscosity is known to influence spectroscopic parameters of organic molecules. In membranes it may vary from a few cP inside the hydrophobic core to 100 cP or above in hydrophilic regions. In the interface regions of the membrane the viscosity depends on the degree of hydration of head groups. Our earlier studies regarding dependence between fluorescence lifetimes of β-glucoside (**II**) and viscosity have shown that at viscosities below 20 cP the viscosity influence is negligible [18]. The anisotropy and fluorescence results suggest that the strong binding of glycoside to the membranes arises from non-specific interactions between the chromanol ring of glucoside and the charged part of choline located in the hydrophilic region of the membrane.

3.4. Calculations of electrostatic potential distribution

In order to deduce the location of the glucoside, we first focus on the data obtained for DL-α-tocopherol (**I**), whose position in a membrane is discussed in the literature. According to the earlier studies [26,27] its location could be due to hydrogen bonding between OH group of the chromanol ring and polar lipid/water interface of the membrane. Some of the researchers suggest that DL-α-Toc molecules reside either in the core region, in an environment of lower values of dielectric constant and viscosity [28–30], or sandwiched between sublayers of a phospholipid bilayer [31,32]. The others locate tocopherol at the interface, where their fluorescence would be quenched by membrane phospholipids [33–35]

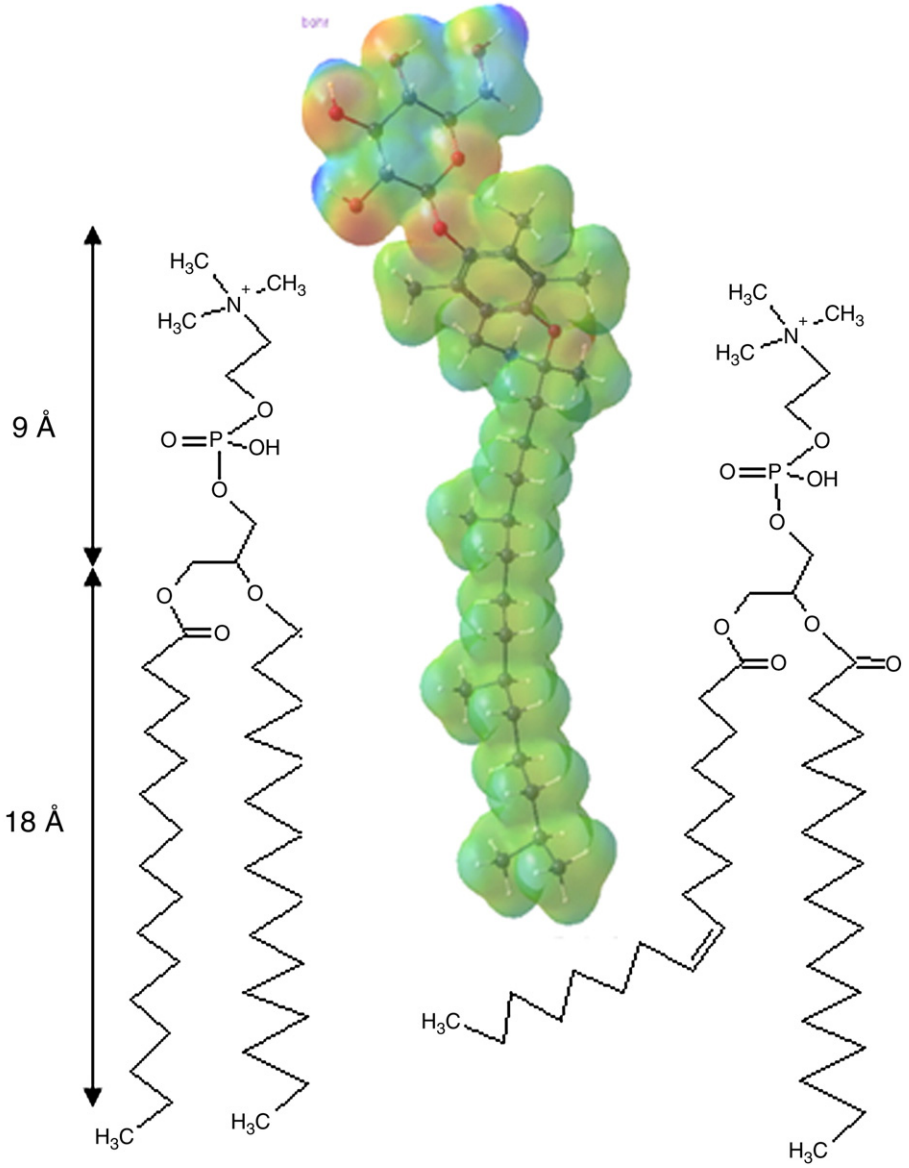

Fig. 3. Schematic estimation of α-Toc β-glucoside (**II**) location in PC and DPPC membrane bilayer. The electrostatic potential distribution was calculated only for glucoside, where green color denotes neutral, red negative and blue positive electrostatic potential value. The chemical structures of PC and DPPC were given for illustrative purpose. All molecules are scaled to this same dimension.

or by hydrogen bond forming between the hydroxyl groups and the microenvironment [16]. The results of our studies suggest that DL-α-Toc (**I**) resides inside the hydrophilic phase of the membrane whereas the phytyl chain is anchored in the hydrocarbon core; such location of D-α-Toc has already been indicated by other authors [36]. It seems that a complex mixture of water, glycerol, carbonyl and methylene groups in hydrophilic part of the membrane creates a polar environment with dielectric constant value close to that found in methanol. Since the investigated glucoside molecule (**II**) is devoid of hydroxyl group, already substituted at 6 positions with glucopyranoside, then the observed strong interaction arises from electrostatic interactions between positively charged nitrogen atom from choline and electric dipole of chromanol ring.

Since the calculation of solvation energies in PC and DPPC membranes with quantum mechanical methods is impossible then we calculated solvation energies of both molecules and its functional groups in three characteristic environments which mimic specific membrane regions where methanol simulates the hydrophilic part and pentadecane the hydrophobic core of the membrane.

In order to estimate contribution to solvation/partition energies from the functional groups in I and II the quantum-chemical calculation was carried out and the results were collected in Table 2. The obtained electrostatic potential distribution and calculated solvation free energies clearly show that glycosidic moiety posses strong affinity for ionic interactions compared to the other parts of the molecule. The results from Table 2 are consistent with thermodynamic data and support our model. In the assumed model the chromanol part is located inside the hydrophilic part of the membrane interacting by electrostatic forces with nitrogen from choline group and the sugar moiety is protruding above the membrane surface. In such case it seems obvious that the presence of a big hydrophilic glucosidic unit interacting strongly with surrounding water molecules prevents a whole glucoside molecule from embedding deeper into the core region of the membrane. Pictorial presentation of the estimated location of glucoside (**II**), presented with electrostatic potential distribution, in both membrane is schematically given in Fig. 3.

4. Conclusions

We have investigated the binding of newly synthesized glycosidic derivative of DL-α-tocopherol embedded into the PC and DPPC phosphatidylcholine membranes. Fluorescence emission, anisotropy and lifetimes were used to estimate the effect of environmental factors on the observed spectral parameters. The intrinsic fluorescence properties were also used to determine quantitatively its partition coefficients into liposomes and calculate thermodynamic parameter of ΔG. Calculated with quantum-chemical methods the solvation energies of (**I**) and (**II**) in simulated membrane environments and electrostatic potential distribution of (**II**) supported the results obtained from spectroscopic studies regarding its location in phospholipid bilayer. The obtained results indicate that the chromanol part of glucoside is located inside the hydrophilic region of the membrane whereas the glucoside moiety extends above the membrane surface. The results indicated the mechanisms responsible for the observed phenomenon, presumably electrostatic interactions between chromanol ring and nitrogen of choline group. As a further extension of the presented results they may be used to explain the reported different physiological activity of tocopheryl glucosides compared to tocopherol [8].

Acknowledgments

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