HAL
open science

Partition of tocopheryl glucopyranoside into liposome membranes studied by fluorescence methods

G. Neunert, P. Polewski, M. Markiewicz, P. Walejko, S. Witkowski, K. Polewski

To cite this version:

G. Neunert, P. Polewski, M. Markiewicz, P. Walejko, S. Witkowski, et al.. Partition of tocopheryl glucopyranoside into liposome membranes studied by fluorescence methods. Biophysical Chemistry, 2009, 146 (2-3), pp.92. 10.1016/j.bpc.2009.10.009 . hal-00605251

HAL Id: hal-00605251

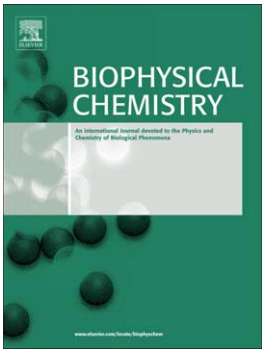
https://hal.science/hal-00605251v1

Submitted on 1 Jul 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript



Partition of tocopheryl glucopyranoside into liposome membranes studied by fluorescence methods

G. Neunert, P. Polewski, M. Markiewicz, P. Walejko, S. Witkowski, K. Polewski

PII: S0301-4622(09)00219-1
DOI: doi: 10.1016/j.bpc.2009.10.009
Reference: BIOCHE 5312

To appear in: *Biophysical Chemistry*

Received date: 28 August 2009
Revised date: 21 October 2009
Accepted date: 27 October 2009

Please cite this article as: G. Neunert, P. Polewski, M. Markiewicz, P. Walejko, S. Witkowski, K. Polewski, Partition of tocopheryl glucopyranoside into liposome membranes studied by fluorescence methods, *Biophysical Chemistry* (2009), doi: 10.1016/j.bpc.2009.10.009

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Partition of tocopheryl glucopyranoside into liposome membranes studied by fluorescence methods

G. Neunert[1], P. Polewski[3], M.Markiewicz[4], P. Walejko[2], S. Witkowski[2], K. Polewski[1*]

[1/] Poznan University of Life Sciences, Department of Physics, 60-637 Poznan, ul. Wojska Polskiego 38/42, Poland
[2/] University of Białystok, Institute of Chemistry, 15-443 Białystok, Al. Pilsudskiego 11/4, Poland
[3/] Poznań University of Technology, Institute of Computing Science, 60-965 Poznań, ul. Piotrowo 5, Poland
[4]Department of Computational Biophysics and Bioinformatics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, 30-387 Kraków, ul. Gronostajowa 7, Poland

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. 1. Scheme 1

* corresponding author

ACCEPTED MANUSCRIPT

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 interactiona 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**) 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).

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 were 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 0,1M phosphate buffer (pH 7,4) and vortexed for 10 min at 50º C (for DPPC) or 22º C (for PC) respectively, until a clear solution was obtained and cooled down to 22º 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 fluorimeter, 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),

* corresponding author

ACCEPTED MANUSCRIPT

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 1x1 cm quartz cuvette; concentrated solutions were measured in 0.3x0.3 cm cuvette at 22 ºC, unless another temperature value is reported in the text.

2.3. Fluorescence steady-state anisotropy measurements

Fluorescence anisotropy measurements were performed using Perkin-Elmer LS-55 fluorometer at 22º 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.

2.4. Quantum-chemical calculations

The energy of tocopherol-glycoside was minimized at 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 298K and 1 atm were calculated at 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 the Table 2. Calculations were carried out using Gaussian 09 program [14], the electrostatic potential was visualized using 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

* corresponding author

ACCEPTED MANUSCRIPT

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 glycoside (**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 indicates the interactions of glycoside with PC and DPPC membranes. The observed increasing intensity of glycoside fluorescence on addition to liposomes confirms its incorporation in the membranes, inset in Fig. 2.

Fluorescence intensity decay of glycoside 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 are similar to these observed in methanol and DMSO/water solution. The monoexponential fluorescence decay suggests that glycoside 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 glycosidic molecules are uniformly distributed in PC and DPPC liposomes at similar sites. Whereas both liposomes differ in hydrophobic core 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.

Table 1.

Our earlier studies on the absorption and emission data and the fluorescence lifetimes of glycoside (**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 glycoside molecule is uniformly distributed in medium value dielectric constant, low viscosity membrane microenvironment.

* corresponding author

ACCEPTED MANUSCRIPT

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 glycoside 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 we may consider as association 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 the equation (1) [19-22]. This equation was used in method described by Huang and Haugland [19].

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

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 glycoside (**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] denotes the water and lipids concentration, respectively.

Figure 2.

The measurements results in DPPC presented as a double reciprocal plot were shown in Fig. 2. Obtained good linearity, as predicted by the equation (1), confirms the presence of interaction between the studied glycoside and PC and DPPC membrane. The value 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 measurements 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 presence of glycoside moiety does not significantly influence the interactions between the membrane and tocopherol as a part of the glycoside molecule. The obtained partition coefficient allowed to calculate standard free energy change ($\Delta G^0 = -RT \ln K_b$) and yields 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 glycoside (**II**) into PC and DPPC membrane.

3.3. Fluorescence anisotropy

* corresponding author

ACCEPTED MANUSCRIPT

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 glycoside content in both membranes. Ranging from 0.05, observed in solvents, to the highest anisotropy value of 0.262 obtained at 3 mol% concentration. This anisotropy value remains constant up to 16 mol% of glycoside 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 membrane 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 reported its maximal anisotropy value [25] what is an indication that tocopherol molecule reside 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 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 was also reported [25]. This phenomenon may be rationalized by the fact that the hydrophilic part in both membranes is this 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-Gly (**II**) and viscosity have shown that at viscosities below 20 cP the viscosity influence is negligible [18]. The anisotropy and fluorescence results suggests that the strong binding of glycoside to the membranes arises from non-specific interactions between chromanol ring of glucoside and charged part of choline located in hydrophilic region of the membrane.

3.4. Calculations of electrostatic potential distribution

* corresponding author

ACCEPTED MANUSCRIPT

In order to deduce the location of the glycoside, 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 suggests that DL-α-Toc molecules reside either in the core region, in an environment of lower values of dielectric constant and viscosity [28-30], 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] 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 investigated glycoside molecule (**II**) is devoid of hydroxyl group, already substituted at 6 position 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 mimics specific membrane regions where methanol simulates hydrophilic part and pentadecane hydrophobic core of the membrane.

Table 2.

In order to estimate contribution to solvation/partition energies from the functional groups in I and II the quantum chemical calculation were carried out and the results are 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

* corresponding author

ACCEPTED MANUSCRIPT

hydrophilic glycosidic unit interacting strongly with surrounding water molecules prevents a whole glycoside molecule from embedding deeper into the core region of the membrane. Pictorial presentation of the estimated location of glycoside (**II**), presented with electrostatic potential distribution, in both membrane is schematically given in Fig. 3.

Figure 3.

4. Conclusions

We have investigated the binding of newly synthesized glycosidic derivative of DL-α-tocopherol embedded into in 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 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 further extension of the presented results they may be used to explain the reported different physiological activity of tocopherol glycosides compared to tocopherol [8].

Acknowledgments

We gratefully acknowledge the partial financial support from grant 508/4 from the Poznan University of Life Sciences and grants N 312 1410 33 and PBZ-KBN-126/T09/2004 from the Polish Ministry of Science and Higher Education.

* corresponding author

ACCEPTED MANUSCRIPT

References

1. X. Wang and P. J. Quinn, Vitamin E and its function in membranes, Prog Lipid Res 38 (1999) 309-336.
2. X. Wang and P. J. Quinn, The location and function of vitamin E in membranes (Review), Molecular Membrane Biology 17 (2000) 143-156.
3. X. Wang and P. J. Quinn, The structure and phase behaviour of alpha-tocopherol-rich domains in 1-palmitoyl-2-oleoyl-phosphatidylethanolamine, Biochimie (rus.) 88 (2006) 1883-1888.
4. J. Atkinson, R. F. Epand and R. M. Epand, Tocopherols and tocotrienols in membranes: A critical review, Free Radical Biology and Medicine 44 (2008) 739-749.
5. M. Lahmann and J. Thiem, Synthesis of a-tocopheryl oligosaccharides, J. Carbohydr.Res. 299 (1997) 23-31.
6. S. Witkowski and P. Wałejko, Synthesis of a-Tocopheryl Glycosides, Zeitschrift fur Naturforschung 56b (2001) 411-415.
7. R. K. Uhrig, M. A. Picard, K. Beureuther and M. Wiessler, Synthesis of antioxidative and anti-inflammatory drugs glucoconjugates, Carbohydrate Research 325 (2000) 72-80.
8. T. Satoh, H. Miyataka, K. Yamamoto and T. Hirano, Synthesis and physiological activity of novel tocopheryl glycosides, Chem Pharm Bull (Tokyo) 49 (2001) 948-953.
9. S. Witkowski and P. Wałejko, Studies in the Acid Catalysed Glycosylation of a-Tocopherol, Zeitschrift fur Naturforschung 57b (2002) 571-578.

* corresponding author

ACCEPTED MANUSCRIPT

10. K. Shimoda, Y. Kondo, K. Abe, H. Hamada and H. Hamada, Formation of water-soluble vitamin derivatives from lipophilic vitamins by cultured plant cells, Tetrahedron Letters 47 (2006) 2695-2698.

11. S. Witkowski, P. Wałejko, M. Knaś, J. Maj, D. Dudzik, J. Marciniak, A. Z. Wilczewska and K. Zwierz, The cleavage of vitamin E galactoside in the rat tissue homogenates, Il Farmaco 59 (2004) 669-671.

12. L. I. Smith, H. E. Ungnade, H. H. Hoehn and S. Wawzonek, The chemistry of vitamin E. VI. The addition of dienes to phenols and hydroquinones, J.Org.Chem 4 (1939) 311-317.

13. A. V. Marenich, C. J. Cramer and D. G. Truhlar, J. Phys. Chem. B 113 (2009) 6378-6396.

14. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. Montgomery, J. A., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, N. J. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. E. Gomperts, O. Stratmann, A. J. Yazyev, R. Austin, C. Cammi, J. W. Pomelli, R. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, Gaussian 09, Revision A 1, Gaussian, Inc.Wallingford CT (2009).

15. C. B. Hübschle and P. Luger, J. Appl. Cryst. 39 (2006) 901-904.

* corresponding author

ACCEPTED MANUSCRIPT

16. J. Kruk, B. Mysliwa-Kurdziel, M. Jemiola-Rzeminiska and K. Strzalka, Fluorescence lifetimes study of alpha-tocopherol and biological prenylquinols in organic solvents and model membranes, Photochem Photobiol 82 (2006) 1309-1314.

17. R. H. Bisby and S. Ahmed, Transverse distribution of alpha-tocopherol in bilayer membranes studied by fluorescence quenching, Free Radic Biol Med 6 (1989) 231-239.

18. G. Neunert, P. Polewski, P. Walejko, M. Markiewicz, S. Witkowski and K. Polewski, Glycosidic moiety changes the spectroscopic properties of DL-a-tocopherol in DMSO/water solution and in organic solvents, Spectrochim Acta A Mol Biomol Spectrosc 73 (2009) 301-308.

19. Z. Huang and R. P. Haugland, Partition coefficients of fluorescent probes with phospholipid membranes, Biochem. Biophys. Res. Commun 181 (1991) 166-171.

20. T. Shyamala and A. K. Mishra, Ground and excited state proton transfer reaction of 3-hydroxyflavone in dimyristoylphosphatidyl-choline liposome membrane, Photochem. Photobiol. 80 (2004) 309-315.

21. O. P. Bondar and E. S. Rowe, Preferential interactions of fluorescent probe Prodan with cholesterol, Biophys J 76 (1999) 956-962.

22. S. Chaudhuri, B. Pahari and P. K. Sengupta, Ground and excited state proton transfer and antioxidant activity of 7-hydroxyflavone in model membranes: Absorption and fluorescence spectroscopic studies., Biophys. Chem. 139 (2009) 29-36.

23. A. F. Sonnen, H. Bakirci, T. Netscher and W. M. Nau, Effect of temperature, cholesterol content, and antioxidant structure on the mobility of vitamin E

* corresponding author

ACCEPTED MANUSCRIPT

constituents in biomembrane models studied by laterally diffusion-controlled fluorescence quenching, J Am Chem Soc 127 (2005) 15575-15584.

24. G. Gramlich, J. Zhang and W. M. Nau, Diffusion of alpha-tocopherol in membrane models: probing the kinetics of vitamin E antioxidant action by fluorescence in real time, J Am Chem Soc 126 (2004) 5482-5492.

25. M. Jemiola-Rzeminska, J. Kruk and K. Strzalka, Anisotropy measurements of intrinsic fluorescence of prenyllipids reveal much higher mobility of plastoquinol than alpha-tocopherol in model membranes, Chem Phys Lipids 123 (2003) 233-243.

26. S. Urano, M. Matsuo, T. Sakanaka, I. Uemura, M. Koyama, I. Kumadaki and K. Fukuzawa, Mobility and molecular orientation of vitamin E in liposomal membranes as determined by 19F NMR and fluorescence polarization techniques, Arch Biochem Biophys 303 (1993) 10-14.

27. S. Urano, M. Kitahara, Y. Kato, Y. Hasegawa and M. Matsuo, Membrane stabilizing effect of vitamin E: existence of a hydrogen bond between alpha-tocopherol and phospholipids in bilayer liposomes, J Nutr Sci Vitaminol (Tokyo) 36 (1990) 513-519.

28. G. Metz, K. P. Howard, W. B. S. van Liemt, J. H. Prestegard, J. Lugtenburg and S. O. Smith, NMR studies of ubiquinone location in oriented model membranes: evidence for a single motionally-averaged population. , J. Am. Chem. Soc. 117 (1995) 564-565.

29. A. Alonso, J. C. Gomez-Fernandez, F. J. Aranda, F. J. F. Bbelda and F. M. Goni, On the interaction of ubiquinones with phospholipid bilayers., FEBS Lett. 132 (1981) 19-22.

* corresponding author

ACCEPTED MANUSCRIPT

30. F. J. Aranda, J. Villalain and J. C. Gomez-Fernandez, A fourier transform infrared spectroscopic study of the molecular interaction of ubiquinone-10 and ubiquinol-10 with bilayers of dipalmitoylphosphatidylcholine., Biochim. Biophys. Acta 861 (1986) 25-32.

31. H. Katsikas and P. J. Quinn, The interaction of coenzyme Q with dipalmitoylphosphatidylcholine bilayers. , FEBS Lett. 133 (1981) 230-234.

32. J. C. Gomez-Fernández, M. A. Llamas and F. J. Aranda, The interaction of coenzyme Q with phosphatydylethanolamine membranes. , Eur. J. Biochem. 259 (1999) 739-746.

33. W. Stillwell, W. Ehringer and S. R. Wassall, Interaction of alpha-tocopherol with fatty acids in membranes and ethanol, Biochim Biophys Acta 1105 (1992) 237-244.

34. V. V. Chudinova, E. I. Zakharova, S. M. Alekseev and R. P. Evstigneeva, [Mechanism of action of vitamin E. Study of the interaction of vitamin E (alpha-tocopherol) and its analogs with fatty acids and their derivatives by fluorimetry], Bioorg Khim 18 (1992) 1528-1534.

35. V. E. Kagan and P. J. Quinn, The interaction of alpha-tocopherol and homologues with shorter hydrocarbon chains with phospholipid bilayer dispersions. A fluorescence probe study, Eur J Biochem 171 (1988) 661-667.

36. B. Samori, G. Lenaz, M. Battino, G. Marconi and I. Domini, On coenzyme Q orientation in membranes: a linear dichroism study of ubiquinones in a model bilayer, J Membr Biol 128 (1992) 193-203.

* corresponding author

ACCEPTED MANUSCRIPT

Figure caption

Figure 1. Chemical structures of DL-α-tocopherol (**I**) and its glycosidic 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.

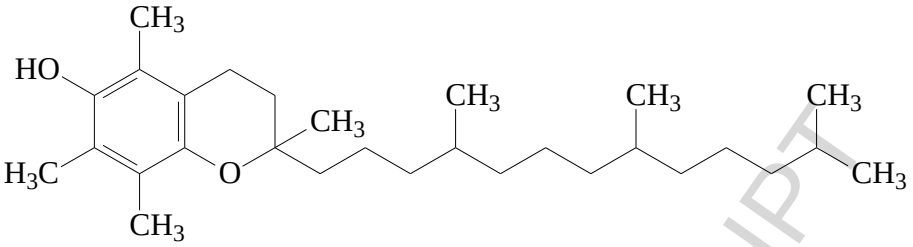
Figure 2. Double reciprocal plot drawn for the calculation of the partition constant K_b characterizing the partition of DL-α-Toc β-glucoside (**II**) into DPPC membrane. The inset shows the original data of fluorescence intensity with increasing concentration of lipid.

Figure 3. Schematic estimation of DL-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.

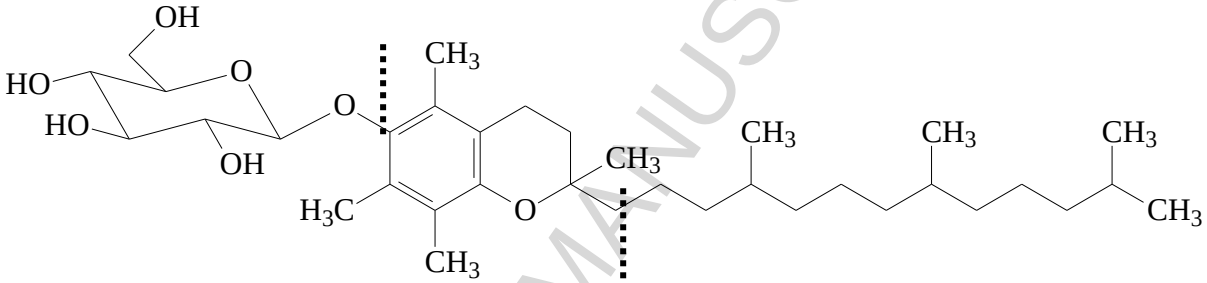
ACCEPTED MANUSCRIPT

Fig. 1

ACCEPTED MANUSCRIPT



I (DL-α-tocopherol)



c b a

II (DL-α-tocopheryl β-glucopyranoside)

ACCEPTED MANUSCRIPT

Fig. 2

ACCEPTED MANUSCRIPT

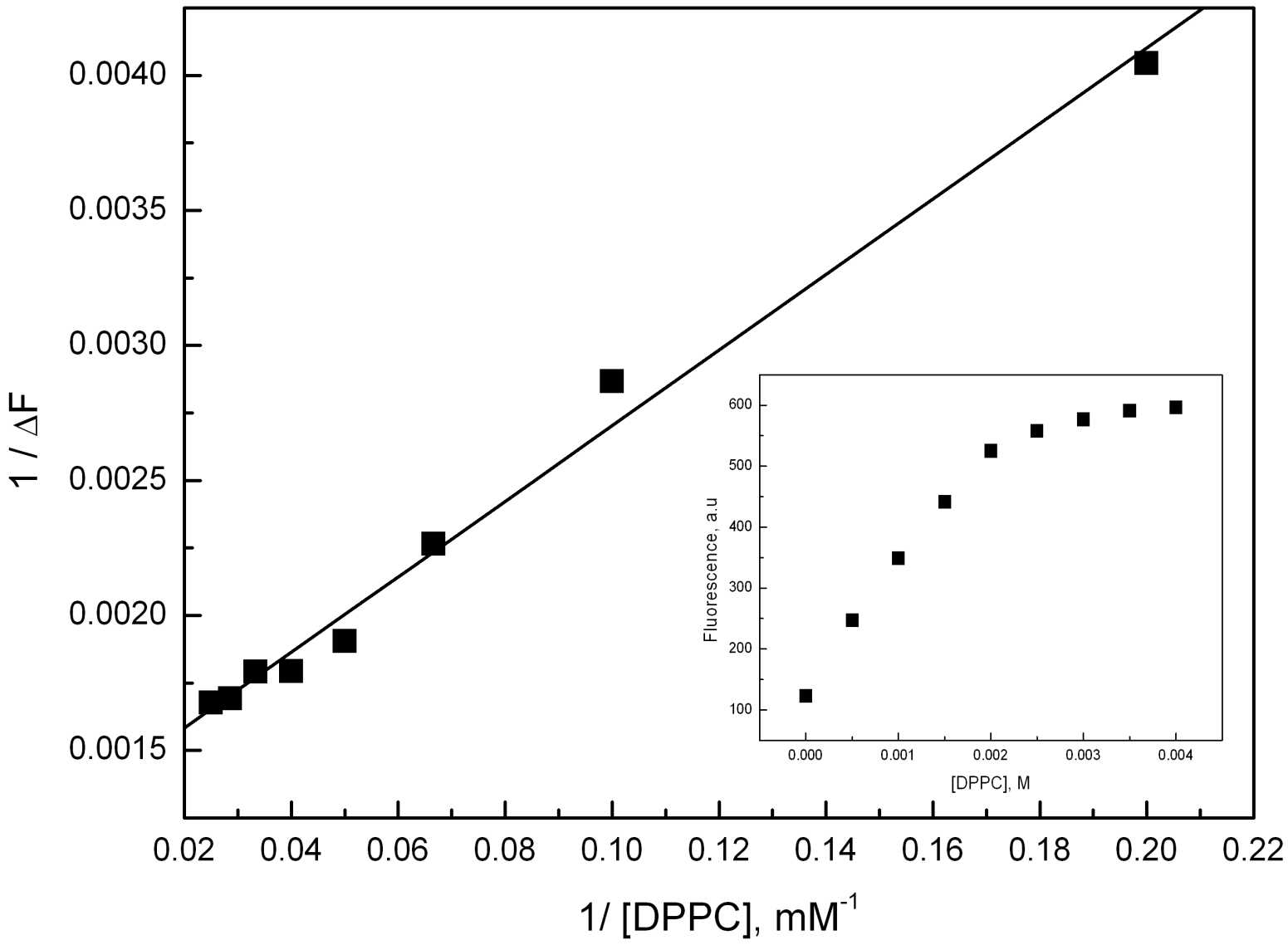
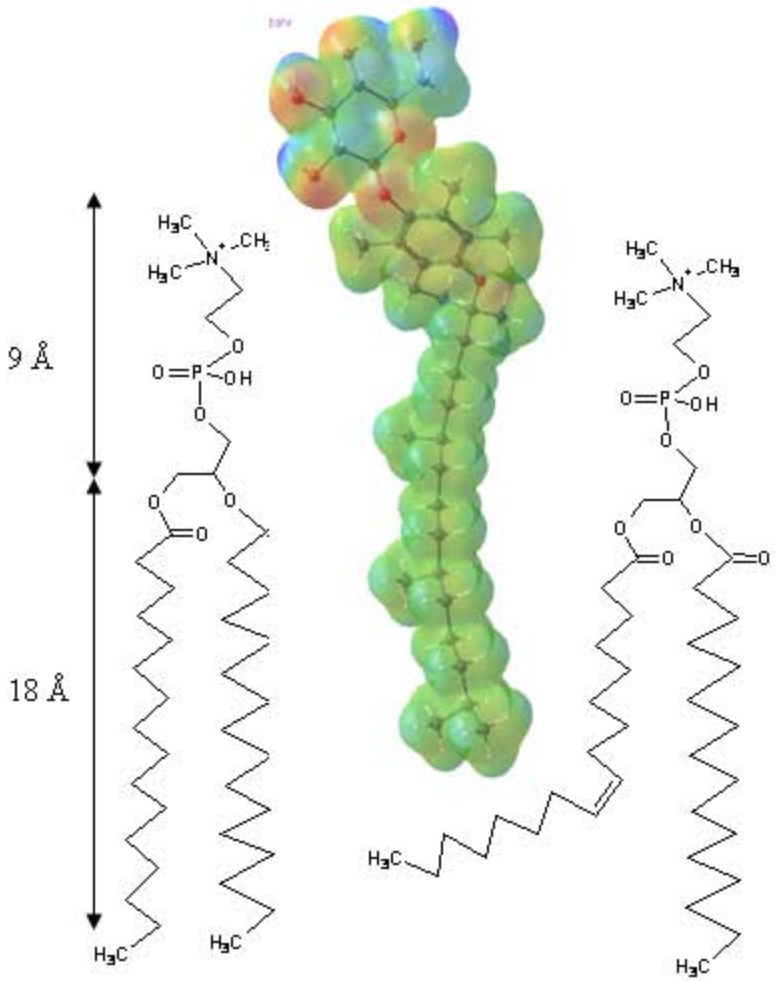


Fig 3

ACCEPTED MANUSCRIPT



ACCEPTED MANUSCRIPT

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. 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 ºC, with excitation wavelength of 290 nm.

	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

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 2. Calculated solvation free energies ΔG^o [kcal/mol] for (I), (II) and functional groups of (II). Details of calculations are described in Section 2.4.

	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

ACCEPTED MANUSCRIPT