Biochimica et Biophysica Acta 815 (1985) 209–214
Elsevier

BBA 72563

Formation of α-tocopherol complexes with fatty acids. Nature of complexes

Alexander N. Erin *, Vasily V. Skrypin and Valery E. Kagan

All-Union Research Center of Mental Health, Zagorodnoe shosse, 2, Moscow, 113152 (U.S.S.R.)

(Received December 12th, 1984)

Key words: α-Tocopherol; Fatty acid; ^{1}H-NMR; Tocopherol-fatty acid complex

Using ultraviolet spectrophotometry and ^{1}H-NMR high-resolution spectroscopy, it has been demonstrated that the formation of α-tocopherol complexes with free fatty acids occurs via two types of interaction, namely (i) formation of a hydrogen bond between the α-tocopherol chromanol nucleus hydroxyl and the carboxyl group of a fatty acid, and (ii) interaction of the fatty acid acyl chains with the chromanol nucleus methyl groups. The second interaction is significantly enhanced by an increase in the number of double bonds in the fatty acid molecule, which results in restriction of the molecular mobility of α-tocopherol. The proposed structural model of α-tocopherol-fatty acid complexes has been confirmed by the use of molecular models. It has been assumed that the efficiency of complex formation of natural tocopherols with fatty acids is correlated with their biological activity.

Introduction

Tocopherols (vitamin E) are indispensable components of the lipid bilayer of biological membranes; a decrease in their content brings about structural and functional damage of the membranes [1]. It is generally known that the stabilizing effect of tocopherols on biological membranes lies in their ability to inactivate free lipid radicals [2], to quench singlet molecular oxygen [3] and to stabilize the membrane lipid bilayer via a Van der Waals interaction with unsaturated fatty acid chains of phospholipids [4,5]. These molecular mechanisms underlying the biological effects of tocopherols have been studied in great detail and are commonly accepted. Recently it has been demonstrated that α-tocopherol can protect biological membranes against the damaging action of free fatty acids [6], whose concentration is sharply elevated under some pathological conditions, such as ischemia, stressory damages, hypoxia, etc. [7–9]. The molecular mechanisms of membrane protection against structural and functional damages induced by fatty acids is their interaction with α-tocopherol with formation of α-tocopherol-fatty acid complexes [10]. The aim of the present work was to investigate the nature of α-tocopherol-fatty acid complexes formed.

Materials and Methods

The ultraviolet absorption spectra of methanol solutions of α-tocopherol, 2,2,5,7,8-pentamethyl-6-hydroxychroman (PMC) and their complexes with fatty acids were recorded on a Perkin-Elmer 555 spectrophotometer. The reference cuvette contained the same amount of fatty acids as the control one. The temperature accuracy during registration of the ultraviolet spectra was ±1°C.

The ^{1}H-NMR high-resolution spectra were recorded on a WH-270 spectrometer (Bruker-Physics) with a B-NC 12 32K computer. All ^{1}H-NMR experiments were run in 5-mm sample tubes.

* To whom correspondence should be addressed.

The sample volume was 1 ml. Residual protonated chloroform ($\delta = 7.25$ ppm) was used as an internal reference. The temperature measurements were made with the standard Bruker variable temperature equipment with accuracy of $\pm 1°C$. The integral intensities and signal amplitudes in the NMR spectra were calculated by a NTCFT (Nicolett) Fourier program with an accuracy of 5–7%. The linewidths of the NMR signals were determined as the ratio of the signal integral intensity to its amlitude.

The results obtained were analyzed statistically on a Hewlett-Packard 85 mini-computer.

The following reagents were used: oleic, linoleic and arachidonic acids and (±)-α-tocopherol (obtained from Serva); palmitic acid and methyl oleate (from ICN Biochemicals); deuterated chloroform (C^2HCl_3) (purchased from Merck). Pentamethylhydroxychroman was kindly supplied by Dr. I.K. Sarycheva (Moscow Institute of Fine Chemical Technology).

Results and Discussion

To identify the functional groups involved in the formation of α-tocopherol-fatty acid complexes, ^{1}H-NMR high-resolution spectroscopy was used. A ^{1}H-NMR high-resolution spectrum of α-tocopherol solution in C^2HCl_3 is shown in Fig. 1. In this spectrum, the NMR line with $\delta = 4.31$ ppm corresponds to the chromanol nucleus hydroxyl proton, the lines with $\delta = 1.76$ and 2.59 ppm correspond to the heterocycle at position 3 and 4, the doublet with $\delta = 2.10$–2.15 ppm to the chromanol nucleus methyl protons, and the line with $\delta = 1.21$ ppm to the phytol chain methyl proton [11].

Addition of fatty acids to α-tocopherol solution causes changes in the ^{1}H-NMR spectra, which can be classified into two types. The first type is a broadening of the α-tocopherol hydroxyl proton ine ($\delta = 4.31$ ppm) after addition of unsaturated and saturated fatty acids to the α-tocopherol solution. The second type of change is a broadening of the chromanol methyl proton doublet ($\delta =$ 2.10–2.15 ppm) and of the phytol chain methylene proton line ($\delta = 1.21$ ppm) as a result of α-tocopherol interaction with only unsaturated fatty acids (Fig. 1).

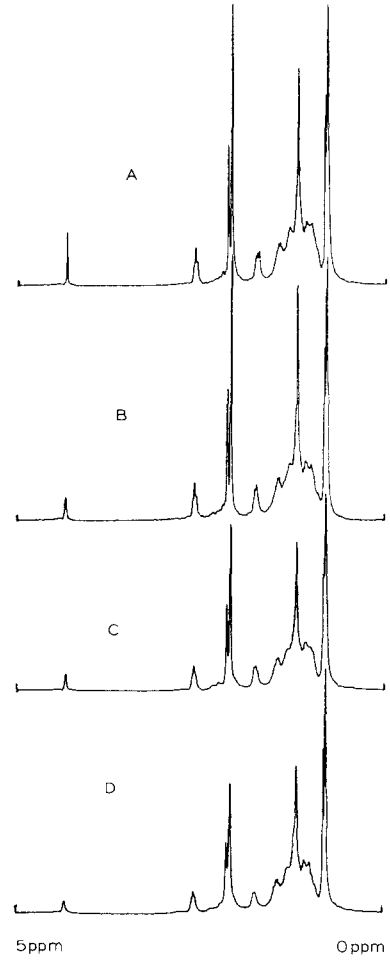

Fig. 1. ^{1}H-NMR high resolution spectrum of C^2HCl_3 solutions containing $5 \cdot 10^{-2}$ M α-tocopherol (A) and α-tocopherol + fatty acid ($5 \cdot 10^{-3}$ M): palmitic (B), linoleic (C) and arachidonic (D) acid. Temperature, 243 K; scan number, 20; pulse delay, 15 s.

The effect of broadening was especially well pronounced in the case of the α-tocopherol hydroxyl line ($\delta = 4.31$ ppm), which is essential for the biological activity of α-tocopherol [12]. This suggests that the hydroxyl group of the chromanol nucleus is involved in the formation of α-tocopherol complexes with both unsaturated (oleic, linoleic, arachidonic) and saturated (palmitic) fatty acids. The K' values for α-tocopherol interaction with fatty acids were determined from the broadening of the hydroxyl proton line ($\delta = 4.31$ ppm)

as:

$$K' = \frac{\Delta\nu_{1/2} - \Delta\nu^0_{1/2}}{\Delta\nu^0_{1/2} \cdot [\text{fatty acid}]}$$

where $\Delta\nu^0)_{1/2}$ and $\Delta\nu_{1/2}$ are the linewidths of the NMR signal ($\delta = 4.31$ ppm) before and after addition of fatty acids to α-tocopherol solution; these values are equal to $(1-3)\cdot 10^3$ M^{-1} and do not depend on the degree of fatty acid saturation. The value of the chemical shift of the NMR signal of the α-tocopherol chromanol nucleus hydroxyl proton in the presence of fatty acids is linearly decreased with an increase in temperature from 243 to 313 K (data not shown), which is typical for the protons involved in the formation of hydrogen bonds [13]. The values of Gibb's free energy, ΔG, which can be calculated from the Arrhenius plot for the temperature dependence of the constant, K', for the fatty acids under study are equal to 3.5–5.5 kcal/mol. These ΔG values are typical for the free energy of a hydrogen bond [14].

The hydrogen bonds may not only be formed via the interaction of the α-tocopherol hydroxyl group with free fatty acids, but with esterified fatty acids containing a C = O group as well. Indeed, the interaction of α-tocopherol with methyl oleate leads to the broadening of the hydroxyl proton NMR line ($\delta = 4.31$ ppm); the determined value of ΔG is practically identical to that for oleic acid.

The broadening of the α-tocopherol ^{1}H-NMR line ($\delta = 2.10$–2.15 ppm) which corresponds to the chromanol nucleus methyl protons at positions 5, 7 and 8 does not depend on temperature but is strongly dependent on the number of double bonds in a fatty acid molecule. During α-tocopherol interaction with unsaturated fatty acids, the width of this signal is significantly increased in the following order: oleic < linoleic < arachidonic acid, while in the case of α-tocopherol interaction with palmitic acid this effect is absent. The broadening of the NMR signal of the phytol chain methylene group protons ($\delta = 1.21$ ppm) also takes place upon α-tocopherol interaction only with unsaturated fatty acids but, unlike the doublet with $\delta = 2.10$–2.15 ppm, is far less dependent on the number of double bonds in an unsaturated fatty acid molecule (Fig. 1).

The type of dependence of the broadening of the NMR doublet of the chromanol nucleus methyl protons ($\delta = 2.10$–2.15 ppm) on the number of double bonds in the fatty acids during their interaction with α-tocopherol is exponential (Fig. 2). Earlier it was shown that α-tocopherol interaction with fatty acids is concomitant with a drop in the absorbance of the α-tocopherol ultraviolet absorption maximum at 210–215 nm; the K values determined from the changes in the ultraviolet absorption spectra increase exponentially, as the number of double bonds in the fatty acid molecule rises [10]. These data correlate well with the broadening of the α-tocopherol ^{1}H-NMR doublet ($\delta = 2.10$–2.15 ppm) upon its interaction with unsaturated fatty acids (Fig. 2). On the other hand, an addition of palmitic acid to α-tocopherol solution at a fatty acid/α-tocopherol molar ratio of 1 : 10 does not result in broadening of the doublet ($\delta = 2.10$–2.15 ppm). This is only natural, since the signifant changes in the ultraviolet absorption spectra upon α-tocopherol interaction with palmitic acid are observed at fatty acid α-tocopherol molar ratios significantly exceeding 10 [10].

The temperature dependence of the K values for α-tocopherol interaction with fatty acids de-

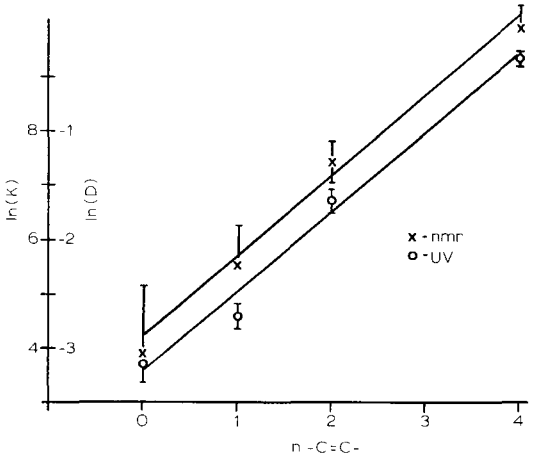

Fig. 2. Dependence of the K value for α-tocopherol interaction with fatty acids as determined from the ultraviolet spectra and for the relative broadening, $D = \frac{\Delta\nu_{1/2} - \Delta\nu^0_{1/2}}{\Delta\nu^0_{1/2}}$, of the chromanol nucleus methyl proton line in the ^{1}H-NMR spectrum at the formation of α-tocopherol-fatty acid complexes on the number of double bonds in a fatty acid molecule ($P < 0.05$).

termined from the ultraviolet absorption spectra appears to be as follows:

$$K = \frac{A_0 - A}{A_0 \cdot [\text{fatty acid}]}$$

where A_0 and A are the absorbances of α-tocopherol at 207–210 nm before and after α-tocopherol addition to the fatty acids. It was found that the K value for α-tocopherol interaction with both saturated and unsaturated fatty acids remains practically unchanged with a rise in temperature from 293 to 313 K (data not shown).

The similarity of the temperature dependence of the broadening of the chromanol nucleus methyl ^{1}H-NMR doublet ($\delta = 2.10$–2.15 ppm) and of the changes in the absorbance of α-tocopherol solutions in the ultraviolet region as well as the exponential increase of these parameters with a rise in the number of double bonds in a fatty acid molecule indicate that the changes in both parameters reflect the same type of interaction in the α-tocopherol-fatty acid complex which is distinct from the hydrogen bond.

Our data demonstrate that the formation of α-tocopherol complexes with fatty acids occurs via two types of interaction, namely, (i) formation of a hydrogen bond between the α-tocopherol chromanol nucleus hydroxyl group and the C = O group of the fatty acid, and (ii) interaction of the acyl chains of fatty acids with the chromanol nucleus methyl groups of α-tocopherol. The latter interaction is markedly enhanced with an increase in the number of double bonds in a fatty acid molecule, which restricts the molecular mobility of α-tocopherol during the complex formation as a result of the broadening in the ^{1}H-NMR signals.

A specific feature of the proposed model of α-tocopherol-fatty acid complex is that the phytol chain of α-tocopherol is not essential for the complex formation. If this model is correct, the complexes will also be formed upon fatty acid interaction with pentamethylhydroxychroman, in which the phytol chain is substituted for by the methyl group. Indeed, the pentamethylhydroxychroman interaction with palmitic acid manifests itself only in the broadening of the hydroxyl proton line with $\delta = 4.00$ ppm. In the course of pentamethylhydroxychroman interaction with linoleic acid, the broadening of the hydroxyl proton with $\delta = 4.00$ ppm is concomitant with the broadening of the doublet ($\delta = 2.29$–2.32 ppm) corresponding to the pentamethylhydroxychroman methyl group protons at positions 5, 7 and 8, but does not affect the NMR signal of methyl groups at position 2 (Fig. 3). The interaction of pentamethylhydroxychroman with these two acids also results in a decrease of the absorbance in the ultraviolet region similar to that observed upon α-tocopherol interaction with fatty acids (Fig. 4). The K values for pentamethylhydroxychroman interaction with palmitic ($K = 60$–70 M^{-1}) and linoleic ($K = (2.8$–$3.0) \cdot 10^3$ M^{-1}) acids determined from the changes in the absorbance are close to those for fatty acid interaction with α-tocopherol.

The hypothetical structure of α-tocopherol-fatty acid complexes has been confirmed by the use of the Pauling-Corey molecular models (Fig. 5). After formation of a hydrogen bond between the linoleic acid carboxyl group and the α-tocopherol hy-

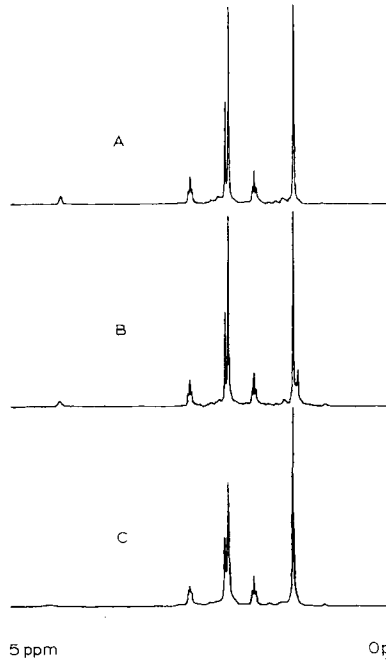

Fig. 3. ^{1}H-NMR high resolution spectra of C^2HCl_3 solutions containing $5 \cdot 10^{-2}$ M pentamethylhydroxychroman (A), pentamethylhydroxychroman $+ 5 \cdot 10^{-3}$ M palmitic acid (B) and pentamethylhydroxychroman $+ 5 \cdot 10^{-3}$ M linoleic acid (C). The ^{1}H-NMR spectra were recorded as indicated in the legend to Fig. 1.

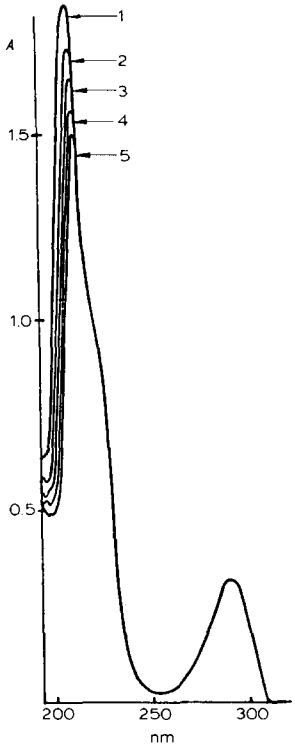

Fig. 4. Ultraviolet absorption spectra of a $5 \cdot 10^{-4}$ M pentamethylhydroxychroman solution in methanol at 293 K before (curve 1) and after addition of increasing concentrations of linoleic acid (curves 2–5). Final concentration of linoleic acid, 10^{-4} M.

droxyl group, the 9,10- and 12,13-*cis*-double bounds of fatty acids form a structure which is complementary to the chromanol nucleus methyl groups. A similar structure is formed when fatty acids are bound to pentamethylhydroxychroman.

The absence of broadening of the NMR signals

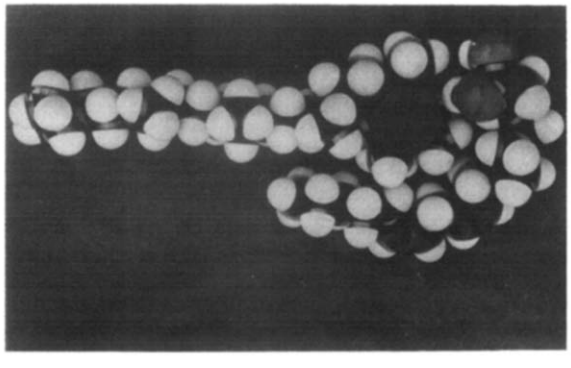

Fig. 5. Molecular model of an α-tocopherol-linoleic acid complex.

of the phytol chain methyl groups in the ^{1}H-NMR spectra upon the formation of α-tocopherol-fatty acid complexes in solution indicates that in the given system no interaction takes place between the *cis*-double bonds of fatty acids with the α-tocopherol phytol chain methyl groups postulated by Lucy and Diplock [4,5]. At the same time, the molecular models do not exclude the possibility of such an interaction. It might therefore be possible that the interaction between the *cis*-double bond of fatty acids and the α-tocopherol phytol chain methyl groups postulated by Lusy and Diplock [4,5] is established in monolayers, model and biological membrane.

The data obtained on the structure of α-tocopherol-fatty acid complexes have led to the following conclusions. Taking into account that the formation of α-tocopherol complexes with fatty acids involves the methyl groups of the chromanol nucleus, one might except that the efficiency of the fatty acid-natural tocopherol complexes will correlate with their biological activity, which, in its turn, depends on the number and localization of the chromanol nucleus methyl groups [12]. It might also be expected that fatty acids will form complexes with synthetic free radical scavengers of the phenolic type, for example, butylated hydroxy toluene, propyl gallate, etc., which prevent some clinical manifestations of vitamin E deficiency [15,16].

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