COMPLEX FORMATION OF IONOL WITH FREE FATTY ACIDS

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In the modern view, a sharp rise in the free fatty acid (FFA) level is an important pathogenetic factor in stress injury, ischemia, and hypoxia [7, 8, 10]. At the cellular level the action of FFA is expressed as damage to biological membranes [7, 10]. It has recently been shown that α-tocopherol protects biological membranes against the harmful action of FFA [5] by forming complexes with them [2]. It has been suggested that the formation of complexes of tocopherols with FFA is one of the molecular mechanisms of the biological action of vitamin E [2]. Many manifestations of avitaminosis-E are known to be preventable by administration of synthetic antioxidants of phenolic type to animals [6, 9]. The aim of this investigation was to study interaction between FFA and ionol (2,6-di-tert-butyl-4-methylphenol), a typical phenolic antioxidant.

EXPERIMENTAL METHOD

Ionol and linoleic acid were obtained from Serva, West Germany, palmitic acid from ICN Biochemicals, USA, and deuterated chloroform from Merck, West Germany.

UV absorption spectra of solutions of FFA and ionol were measured on the Perkin-Elmer 555 spectrophotometer. High-resolution ^{1}H-NMR spectra were recorded on a WH-270 spectrometer (Brucker, West Germany). As the internal standard, the signal of chloroform (α = 7.25 PPM) was used. The spectra were analyzed in accordance with the NTCFT program (from Nicolet, USA).

EXPERIMENTAL RESULTS

It was shown previously that complex formation by FFA with α-tocopherol is accompanied by a change in absorption of α-tocopherol in the UV region at 210-215 nm [2]. As our experiments showed, the introduction of increasing concentrations of linoleic acid into a solution of ionol in methanol leads to a decrease in optical density in the 207-210-nm region but does

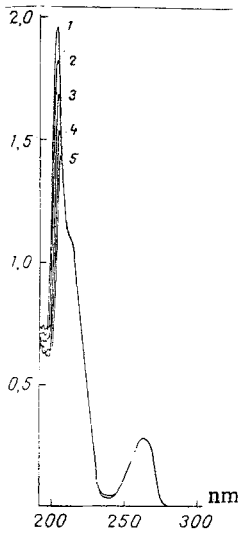

Fig. 1. UV absorption spectra of ionol in methanol before (1) and after (2-5) addition of increasing amounts of linoleic acid to the solution. Final concentration of acid (5) 10^{-4} M, of ionol 1.5 × 10^{-4} M. 20°C.

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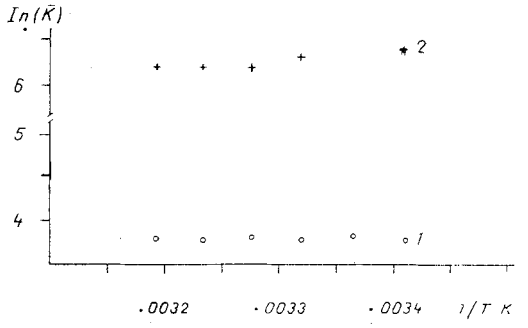

Fig. 2. Dependence of constant of interaction of ionol with palmitic (1) and linoleic (2) acids on temperature plotted in Arrhenius coordinates.

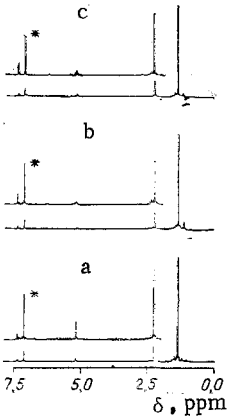

Fig. 3. High-resolution ^{1}H-NMR spectra of solutions of: a) ionol (5×10^{-2} M), b and c) ionol in presence of palmitic (5×10^{-3} M) and linoleic (5 10^{-3} M) acids respectively. Asterisks indicate regions of the same spectra, magnified vertically by four times. Spectra recorded at 243°K.

not change the optical density at the 275-nm peak (Fig. 1). Similar spectral changes were observed after addition of palmitic acid to the ionol solution. Changes in optical density after addition of FFA to ionol solution were observed virtually instantaneously and they were proportional to the FFA concentration. With this in mind, equilibrium constants of interaction of ionol with FFA can be calculated. For an equilibrium system we have:

$$\text{IONOL} + \text{FFA} \underset{K_{-1}}{\overset{K_{+1}}{\rightleftharpoons}} \text{COMPLEX}$$

Hence

$$K = \frac{K_{+1}}{K_{-1}} = \frac{[\text{COMPLEX}]}{[\text{IONOL}]\cdot[\text{FFA}]} = \frac{A_0 - A}{A_0 \cdot [\text{FFA}]},$$

where A_0 and A denote optical densities of ionol solutions at 207 nm respectively before and after addition of FFA.

The equilibrium constants of interaction of ionol with palmitic and linoleic acids, calculated in the manner shown above, were 47.2 ± 1.5 M^{-1} and $(0.91 \pm 0.07) \times 10^3$ M^{-1} respectively. These values are close to those of the constants of formation of complexes of these fatty acids with α-tocopherol [2]. The values of constants of interaction of ionol with both saturated palmitic acid and unsaturated linoleic acid, calculated from changes in the UV spectra, are virtually independent of temperature (Fig. 2).

To identify groups of atoms taking part in interaction between ionol and FFA, the method of high-resolution ^{1}H-NMR spectroscopy was used. The high resolution spectrum of ionol solution in deuterated chloroform is shown in Fig. 3. In the spectrum the signal with $\delta = 1.42$ ppm corresponds to protons of tert-butyl groups, the signal with $\delta = 2.27$ ppm to a proton of

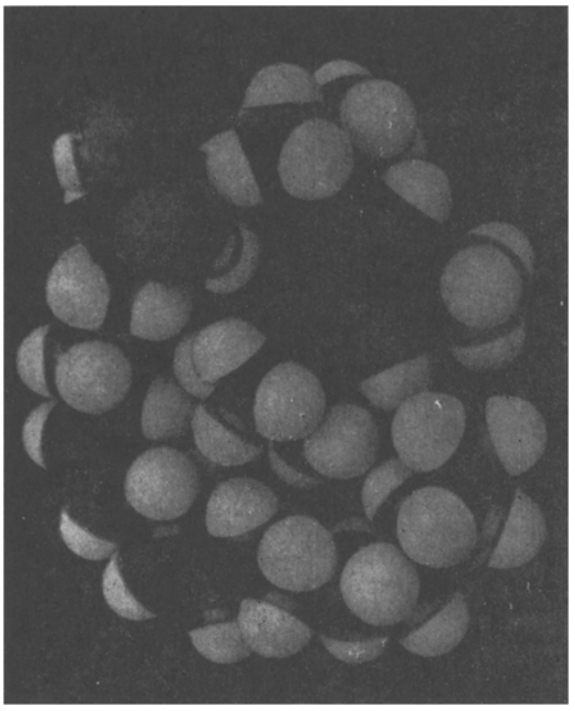

Fig. 4. Molecular model of complex of ionol with linoleic acid

methyl group, the signal with δ = 5.16 ppm to a proton of the OH group, and the signal with δ = 6.96 ppm to protons of the ring in positions 3 and 5.

On the addition of linoleic acid to the ionol solution, widening of the signal of the OH group and of protons of tert-butyl groups of ionol was observed (Fig. 3). This conclusion is based on comparison of integral intensities and amplitudes of signals in the ^{1}H-NMR spectra. Integral intensities of signals remained unchanged, but amplitudes were reduced, evidence of widening of the signals.

The signal of the OH-group, the presence of which determines the properties of the phenolic inhibitor as an antioxidant [6], was widened the most. This widening of the signal with δ = 5.16 ppm is evidence that OH-groups participate in the formation of complexes of ionol with FFA. This widening was observed during interaction of ionol both with unsaturated linoleic and with saturated palmitic acids. Constants of interaction of ionol with FFA, calculated from widening of the signal of the OH-group (δ = 5.16 ppm) by the equation:

$$K = \frac{\Delta\nu_{1/2} - \Delta\nu^{\circ}_{1/2}}{\Delta\nu^{\circ}_{1/2} \cdot [\text{FFA}]},$$

where $\Delta\nu^{\circ}_{1/2}$ and $\Delta\nu_{1/2}$ are half-widths of signals with δ = 5.16 ppm before and after addition of FFA to the ionol solution respectively, have values of about $1.5\text{-}3.0 \times 10^3$ M^{-1}, and do not depend on the degree of unsaturation of the FFA. These values are very close to those of constants of formation of the hydrogen bond during interaction of FFA with α-tocopherol [1]. The value of the free Gibbs' energy (ΔG) can be calculated from the graph of constant K' as a function of temperature in Arrhenius coordinates, and for palmitic and linoleic acids they were 7-9 kcal/mole, close to the value of ΔG of the hydrogen bond [3]. The magnitude of the chemical shift of the proton of the ionol OH-group in the presence of FFA decreased linearly with an increase of temperature from 243 to 313°K, which also is characteristic of protons participating in hydrogen bond formation [4].

Widening of the signal of the NMR-spectrum of ionol with δ = 1.42 ppm, corresponding to protons of tert-butyl groups, was observed on addition of the unsaturated linoleic acid to the ionol solution, but was virtually not observed during interaction of ionol with palmitic acid (Fig. 3). This widening, like changes in absorption in the UV region accompanying interaction of ionol with FFA, was virtually independent of temperature between 243 and 313°K.

It can be tentatively suggested that the similar characters of temperature dependence of widening of the signal of tert-butyl groups in the NMR spectrum and changes in absorption in the UV region of ionol solution, and also the sharp increase in these parameters with an increase in the degree of unsaturation of the FFA are evidence that changes in both these parameters reflect the same type of interaciton in the FFA–ionol complex, distinct from a hydrogen bond.

It can thus be concluded from all the facts described above that the formation of complexes of ionol with FFA takes place on account of two types of interactions: 1) the hydrogen bond of the OH-group of ionol with the carboxyl group of FFA; 2) interactions of the hydrocarbon chain of FFA with the tert-butyl groups of ionol. This latter interaction is sharply intensified during the formation of complexes of ionol with unsaturated fatty acids, as reflected in an increase in the constant of interaction calculated from changes in UV spectra.

The suggested structure of complexes of ionol with FFA is confirmed by molecular models (Fig. 4): During hydrogen bond formation between the carboxyl group of linoleic acid and the OH-group of ionol, the 9,10- and 12,13-cis-double bonds of FFA form a structure complementary to the tert-butyl groups of ionol.

It was shown previously that the formation of complexes of α-tocopherol with FFA also takes place on account of two types of interactions: hydrogen bond formation and van der Waals interactions of cis-unsaturated fatty acids with methyl groups of the chromane ring [1]. These data, and also the results of the present investigation, lead to the conclusion that the formation of complexes of this kind must also be observed with other phenolic antioxidants, containing methyl groups in their aromatic ring.

The high values of constants of interaction of ionol with FFA in solutions suggest that complex formation can take place quite effectively in biological membranes also.

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