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Kinetics of bimolecular decay of α-tocopheroxyl free radicals studied by ESR

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The kinetics of bimolecular decay of α-tocopheroxyl free radicals ($T^{\cdot}$) was studied by ESR mainly in ethanol and heptanol solvents. A second-order kinetic law was observed during the whole course of reaction ($-d[T^{\cdot}]/dt = 2k[T^{\cdot}]^2$) and the following rate constants were determined with good accuracy in the temperature range 281–321 K: ethanol: $\log(2k) = 8.2 \pm 0.5 - (6.6 \pm 0.7$ kcal/mol)/$(2.3RT)$ $M^{-1} \cdot s^{-1}$; heptanol: $\log(2k) = 6.1 \pm 0.4 - (4.3 \pm 0.6$ kcal/mol)/$(2.3RT)$ $M^{-1} \cdot s^{-1}$. The global rate constant clearly increases with solvent polarity.

α-Tocopheroxyl; Bimolecular decay; Kinetics; Solvent effect; ESR

1. INTRODUCTION

Vitamin E (α-tocopherol or TH) appears to be the major lipid-soluble antioxidant in biological systems [1]. The prevention of the auto-oxidation of an organic compound RH by hindered phenols like vitamin E is a free radical process which is now well understood [2,3]. In this process, the antioxidant (TH) primarily transforms chain carriers of oxidation, very active peroxyl free radicals, into α-tocopheroxyl ($T^{\cdot}$) free radicals:

$$ROO^{\cdot} + TH \longrightarrow ROOH + T^{\cdot} \quad (1)$$

which are unable to carry any chain.

Vitamin E appears to be very efficient when compared with most of synthetic antioxidants [4]. It has been shown that some of its superior properties are connected to the lack of reactivity of α-tocopheroxyl free radicals with oxygen in reaction 2:

$$T^{\cdot} + O_2 \longrightarrow \text{radical products} \quad (2)$$

and to their low reactivity in the chain transfer (reaction 3) and in the bimolecular self-reaction (reaction t) [1,4,9,10]:

$$T^{\cdot} + RH \longrightarrow TH + R^{\cdot} \quad (3)$$

$$T^{\cdot} + T^{\cdot} \longrightarrow \text{non-radical products} \quad (t)$$

The nature and kinetic features of termination steps such as reaction t are of prime importance for understanding the role of vitamin E in the prevention of auto-oxidation of unsaturated fatty acids and to account for the synergistic effects of vitamin C and/or of amino acids [4] on the inhibitory properties of vitamin E.

Few rate constant values of reaction t have been reported and there are discrepancies as to the orders of magnitude [5–9].

The object of the present work is to study the kinetics of bimolecular decay of α-tocopheroxyl free radicals – process t – in solvents of different polarity by ESR. Solvents of intermediate polarity have been selected – methyl heptanoate, heptanol, ethanol – mainly for their ability to dissolve both vitamins C and E.

2. MATERIALS AND METHODS

α-Tocopherol (puriss), heptanol (puriss) and α,α′-diphen-

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ylpicrylhydrazyl or DPPH (pract.) were from Fluka. Ethanol was a rectapur Prolabo product.

Reaction of DPPH with α-tocopherol was studied on an Applied Photophysics model 1705 stopped-flow spectrometer. Consumption of DPPH was monitored by visible spectrophotometry at 520 nm (where α-tocopheroxyl free radicals do not absorb).

ESR spectra of α-tocopheroxyl free radicals were recorded on a Bruker ER 200 D spectrometer with a field modulation of 2.5 Gpp. The temperature cavity was controlled by a Bruker ER 4111 VT temperature control unit.

Reactants (α-tocopherol and DPPH) were first preheated to the temperature of experiment; then the ESR quartz tube was filled with the mixture and transferred into the ESR cavity.

ESR spectra were recorded and doubly integrated on a Nicolet Nic80 calculator. The calibration sample was DPPH. ESR spectra were doubly integrated under the same experimental conditions.

3. RESULTS

α-Tocopheroxyl (T$^{\cdot}$) free radicals are very stable in the presence of oxygen. In a non-degassed but concentrate solution of α-tocopherol, a residual signal of T$^{\cdot}$ can be observed by ESR (fig.1). However, under our experimental conditions, no residual signal can be detected in the 3×10^{-4} M solution of α-tocopherol. We consequently tried to obtain high initial concentrations of T$^{\cdot}$ by adding DPPH to non-degassed solutions of TH.

Using the stopped-flow technique, we first monitored DPPH consumption at 520 nm, and verified that the reaction:

$$TH + DPPH^{\cdot} \longrightarrow T^{\cdot} + DPPH_2 \qquad (4)$$

is fast [6]. As α-tocopheroxyl free radicals absorb light in the visible spectrum with a maximum at 425 nm [5,6], we were able to check the production and consumption of T$^{\cdot}$ in the mixture.

The rates of disappearance of T$^{\cdot}$ free radicals were measured in non-degassed solvents by monitoring the ESR spectrum of T$^{\cdot}$ free radicals. The initial concentrations were: $[DPPH]_0 = 2 \times 10^{-4}$ M, $[TH]_0 = 3 \times 10^{-4}$ M, and the temperature range was 281–321 K. The time which elapsed between the mixing of reactants and the beginning of recording operations corresponded to full consumption of DPPH (no contribution of DPPH in the ESR signal was ever observed in the spectrum of T$^{\cdot}$). A typical decay curve of T$^{\cdot}$ free radicals is shown in fig.2. From such a curve it is possible to calculate a second-order rate constant k of reaction

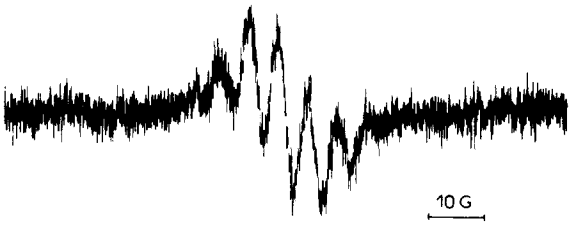


Fig.1. ESR spectrum of α-tocopheroxyl free radicals at room temperature (field modulation 2.5 Gpp) in a non-degassed solution of heptanol ($[\alpha\text{-TH}] = 1.08 \times 10^{-2}$ M).

t with the rate law:

$$-d[T^{\cdot}]/dt = 2k[T^{\cdot}]^2$$

This rate constant correctly fits the decay curve throughout the course of reaction. Under these conditions, process t is irreversible. We also made few measurements in methyl heptanoate as solvent. Rate constants are still second order but at initial times only. In this case, precision in the determination of k is poorer. This phenomenon is probably due to the fact that process t is no longer irreversible (cf. [3] for a discussion on this point).

The influence of temperature is shown in fig.3. From this plot, we calculate the activation energies

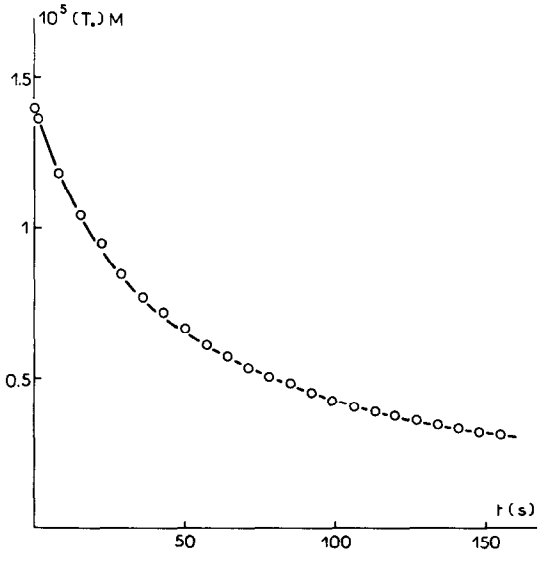


Fig.2. Plot of α-tocopheroxyl free radical decay as a function of time at 290 K in ethanol. $[\alpha\text{-TH}]_0 = 3 \times 10^{-4}$ M, $[DPPH]_0 = 2 \times 10^{-4}$ M. (○) Experimental data; (–) theoretical second order decay curve, with $k = 809$ M$^{-1} \cdot$s^{-1}.

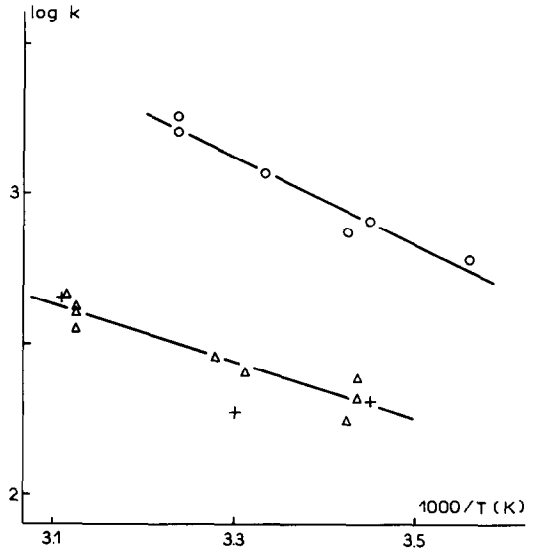


Fig.3. Influence of temperature on rate constant (k) of bimolecular decay of α-tocopheroxyl free radicals: (○) in ethanol, (△) in heptanol, (+) in methyl heptanoate.

and pre-exponential factors:

ethanol: $\log(2k) = 8.2 \pm 0.5 - (6.6 \pm 0.7 \text{ kcal/mol})/(2.3RT)$ $M^{-1} \cdot s^{-1}$

heptanol: $\log(2k) = 6.1 \pm 0.4 - (4.3 \pm 0.6 \text{ kcal/mol})/(2.3RT)$ $M^{-1} \cdot s^{-1}$

Although they are more dispersed, the rate constant values obtained in methyl heptanoate seem to be slightly lower than those in heptanol.

The ESR technique which is presently described could not be used for measurements in benzene because the rate of process 4 is too fast.

4. DISCUSSION

α-Tocopheroxyl free radicals appear to be very stable in the presence of oxygen, since we detect an ESR signal in non-degassed solutions at high concentration of α-tocopherol. This observation was first made by Doba et al. [9] and by us [4]. These radicals are also remarkably stable in the absence of oxygen, since their second-order decay rate constant is exceptionally low, 10^2–10^3 $M^{-1} \cdot s^{-1}$ at room temperature, which should be compared with 10^8–10^9 $M^{-1} \cdot s^{-1}$ for recombinations of conventional free radicals. Moreover, this rate constant clearly decreases with solvent polarity, from ethanol to heptanol, as shown by the following values:

ethanol: $2k = 1400$ $M^{-1} \cdot s^{-1}$ (20°C) [this work]
heptanol: $2k = 560$ $M^{-1} \cdot s^{-1}$ (20°C) [this work]

chloroform: $2k = 190$ $M^{-1} \cdot s^{-1}$ (room temperature) [5]
benzene: $2k = 880$ $M^{-1} \cdot s^{-1}$ (room temperature) [5]
cyclohexane: $2k = 350$ $M^{-1} \cdot s^{-1}$ (room temperature) [7]
benzene: $2k = 0.061$ $M^{-1} \cdot s^{-1}$ (room temperature) [8]
benzene: $2k = 3000$ $M^{-1} \cdot s^{-1}$ (23°C) [9]

Literature data concern various solvents. Although values obtained in benzene are somewhat erratic [5,8,9], the orders of magnitude are similar if we exclude the data from [8]. In the latter work, a contribution of 'residual' α-tocopheroxyl free radicals to the ESR signal is probable.

Boguth and Niemann [5] find that k decreases by a factor of 4 when solvent polarity increases from benzene to chloroform. This is contrary to our observations in heptanol and ethanol. This divergence could be due to the technique of optical absorption used by these authors and to trace impurities in the solvents.

In the literature, we found no data concerning the influence of temperature on k. In the gas phase (apolar medium), rate constants for recombination are in the range 10^9–10^{10} $M^{-1} \cdot s^{-1}$ with zero activation energies; rate constants for disproportionation are generally in the range 10^6–10^8 $M^{-1} \cdot s^{-1}$ with a small activation energy (2–3 kcal/mol). The present results obtained in heptanol and methyl heptanoate (the least polar solvents) are consistent with these facts. Moreover, both pre-exponential factor and activation energy increase with solvent polarity. This fact is generally interpreted in terms of an activated complex which is less solvated than the reactants.

The self reaction of α-tocopheroxyl free radicals has been reported to give α-tocopherol and o-quinone methide by disproportionation [11–13]. This result is also in accordance with the non-zero activation energy which we find for k.

Some comparisons can be made with other

hindered phenolic free radicals. From a recent review by Denisov and Khudyakov [3], disproportionation rate constants for 2,6-di(*t*-butyl)-4-R-phenoxyl free radicals, with R having a mobile hydrogen atom, are in the range 10^3–10^4 $M^{-1} \cdot s^{-1}$. For dimethyl-α-chromanoxyl, which is similar to α-tocopheroxyl, Roginsky et al. [14] propose a value for the rate constant of 1100 $M^{-1} \cdot s^{-1}$. These figures agree with ours.

In conclusion, the kinetics of bimolecular decay of α-tocopheroxyl free radicals have been studied by ESR. Rate constant values have been measured at different temperatures in methyl heptanoate, heptanol and ethanol solvents. The bimolecular decay of $T^{\cdot}$ is probably a disproportionation process. Its rate constant markedly depends on solvent polarity. In all the studied solvents, the rate constants are found to be exceptionally low which partly accounts for the excellent antioxidant efficiency of vitamin E.

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