The effect of aromatic amines and phenols in the thiyl-induced reactions of polyunsaturated fatty acids

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HIGHLIGHTS
- LH micelles were used for the parallel study of peroxidation/cis–trans isomerization.
- Both 2-mercaptoethanol and diphenylamine alone protect LH from oxidation.
- Aminyl radicals promote thiyl-radical-induced cis–trans isomerization of LH in air.

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ABSTRACT
Thiols are well known for their role in cellular redox homeostasis, while aromatic amines and phenols are the best known classes of chain-breaking antioxidants. On the other hand, thiyl radicals are known to catalyse the double bond isomerization in PUFA. We investigated the role and interplay of 2-mercaptoethanol and diphenylamine in the parallel processes of peroxidation and cis–trans isomerization of linoleic acid (LA) during gamma radiolysis, both in solution and micelles. Both compounds, used alone were able to protect LA from oxidation; however pro-oxidant activity and enhanced isomerization was observed when they were used together, depending on the experimental settings. Instead, α-tocopherol protected LA from both oxidation and isomerization in the presence of thiols under any tested settings. The mechanistic scenario is discussed highlighting the role of diphenylaminyl radicals in promoting thiyl-radical-induced cis–trans isomerization in the presence of oxygen.

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1. Introduction

Due to the crucial roles of lipids in structural and signaling activities, the control of lipid reactivity and transformations is an interdisciplinary research field extending over chemistry to biology and medicine (Chatgilialoglu et al., 2014; Halliwell and Gutteridge, 2007). In this context, the protection against their degradation under oxidative and free radical conditions is of special interest. The reactions of polyunsaturated fatty acids (PUFA) with free radicals are known to occur via two main processes: (i) lipid peroxidation (Niki, 2012) and (ii) cis–trans isomerization (Lykakis et al., 2015; Ferreri and Chatgilialoglu, 2012). The mechanism and products of each process have been studied extensively and are now fairly well documented and understood. Scheme 1 shows the interplay of the two processes in the case of linoleic acid moiety and the corresponding main products. The initial step of peroxidation is hydrogen abstraction from the bisallylic position, which can be performed by a variety of radicals, followed by the reaction with oxygen. Conjugated diene hydroperoxides having the trans,cis double bond geometry are the initial stable products (Yin et al., 2011). In free radical isomerization, the addition–elimination of a thiyl radical is enough to produce mono-trans geometrical isomers (Chatgilialoglu and Ferreri, 2005). An overall damaging potential is produced, that must be carefully considered for its consequences in a biological scenario, since peroxidation is a chain reaction (Yin et al., 2011) and isomerization is a catalytic process (Ferreri et al., 2015).

Abbreviations: ArOH, α-tocopherol; LH, Linoleic acid; PUFA, Polyunsaturated Fatty Acids; PhNO, diphenylnitroxy radicals; LOOH, Lipid hydroperoxide; PB, phosphate buffer; RS, thiyl radicals; Ψ, volume part of the solvent in a solvent mixture
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investigated the combined role of Ph2NH and HOCH2CH2SH in the biomimetic model systems consisting of the micellar solution of LH in the presence of thiols and molecular oxygen, contributing to a better understanding of complex interactions related to free radical stress and antioxidant control, as well as of the mechanisms of aromatic amines toxicity.

2. Materials and methods

Unless otherwise noted, solvents and chemicals were of the highest grade commercially available (Aldrich, Fluka, Sigma) and were used as received.

Initially γ-irradiation of phosphate buffered air-equilibrated or N2O-saturated solutions Ψ (EtOH: H2O)=(1:1) (pH 5) containing 0.5 mM of LH and TWEEN®-20 (a non-ionic surfactant soluble in ethanol/water), in the presence and absence of HOCH2CH2SH was studied (Mihaljević et al., 2011). After 100 Gy of irradiation, lipid components were extracted with a solvent mixture Ψ (CH2Cl2:MeOH)=(2:1), deaerated and analyzed for quantitative determination of LOOH by the spectrophotometric ferric thiocyanate method, as described earlier (Mihaljević et al., 1996). In parallel the analysis of trans fatty acid formation at 100 Gy was carried out by GC, after treatment of the lipid extracts with an ethereal solution of CH2N2 in order to transform quantitatively the free fatty acid into the corresponding methyl ester. In the absence of thiols no trace of trans fatty acid was detected. By replacing TWEEN®-20 with another non-ionic surfactant Brij 35, similar results were obtained (Mihaljević et al., 2011).

Next, model system containing mixed surfactant micelles and buffer was prepared by slow solubilization of LH in non-ionic surfactant micelles previously formed by mixing TWEEN -20 or Brij 35 and NaH2PO4 (PB), pH 6.5. The composition of the investigated systems was typically 0.5 mM LH, 0.28 mM TWEEN-20 and 5 mM PB (pH--5). Gamma radiolysis of the solutions with typical composition and in the presence of 2.8 mM HOCH2CH2SH and/or 83 μM Ph2NH, which was added prior to irradiation, was performed. It should be pointed out that solubility of Ph2NH in micellar solutions is much lower than in homogeneous solutions so its concentration had to be reduced to 83 μM.

Radiolysis was performed at room temperature using panoramic 60Co source at dose rate 2.4 and 274.8 Gy min⁻¹. The “transit time” (the time during the movement of the source to and from irradiation position) was 2.2 s, corresponding to the transit irradiation dose of about 0.10 Gy at the dose rate of 2.4 Gy min⁻¹ and 10 Gy at the dose rate of 274.8 Gy min⁻¹, which had to be taken into consideration at doses lower than 100 Gy. Micelles were irradiated in equilibrium with air or after saturation with N2. After irradiation, lipid components were extracted with a solvent mixture of Ψ (CH2Cl2:MeOH)=(2:1), deaerated by nitrogen, and an aliquot of the sample was taken out from the lower layer for the quantitative determination of LOOH. All further analysis was carried out in three independently prepared solutions. The concentration of LOOH was determined by the spectrophotometric ferric thiocyanate method following a published procedure (Mihaljević et al., 1996), using UV/vis spectrophotometer Varian Cary 4000. The rest of the lipid extract was used for GC analysis of geometrical isomers using the known conditions for the separation of cis and trans isomers (Ferreri et al., 2001; Mihaljević et al., 2011; Chatgilialoglu et al., 2002; Chatgilialoglu et al., 2005). In order to transform linoleic acid and its geometrical isomers into the corresponding methyl esters the re-action solutions were treated with an ethereal solution of diazomethane (Glastrup 1998). A Varian 450 gas chromatograph equipped with a flame ionization detector and a Rtx-2330 (90% biscyanopropyl/10% phenylcyanopropylpolysiloxane capillary column;

![Scheme 1. Radical-based peroxidation and cis–trans isomerization processes of linoleic acid.](image-url)
105 m x 0.25 mm) was used with the following oven program: temperature started from 180 °C, held for 35 min, followed by increase of 10 °C min⁻¹ up to 250 °C and held for 5 min. Methyl esters were identified by comparison with the retention times of authentic samples, which are commercially available.

3. Results and discussion

3.1. Irradiation of linoleic acid in homogeneous solutions

LH is soluble in Y (EtOH:H₂O) = (1:1). The γ-radiolysis of ethanol/water led to the transient species shown in Eqs. (1) and (2), where R⁻ represent the produced alcohol radicals (i.e., *CH₂CH₂OH, CH₃CHOH, CH₃CH₂O*) (Asmus et al., 1973). Solvated electrons (e⁻ sol⁻) in N₂O-saturated solutions are transformed into HO⁻ radical [Eq. (3)]. Hydrogen abstraction from ethanol by HO⁻ radical and H⁺ atoms increases the production of alkyl radicals [Eq. (4)]. Alkyl radicals react with HOCH₂CH₂SH to give the corresponding thiyl radicals [Eq. (5), k₅ = 2 x 10⁷ M⁻¹ s⁻¹] (Buxton et al., 1988; Ross et al., 1998). In O₂-atmosphere alkyl radicals give alkylperoxyl radicals [Eq. (6)] (Porter, 1986), which in the presence of HOCH₂CH₂SH couple. One possible explanation for the observed loss of protective activity of aromatic amines towards the oxidation/isomerization of LH in the presence of HOCH₂CH₂SH is the occurrence of chain transfer/propagation reactions caused by amyl radical [Eq. (11)]. Based on the calculated gas phase S–H Bond Dissociation Enthalpy (BDE) for thiols (e.g. cysteine BDE = 87.3 kcal/mol) (Roux et al., 2010), and diphenylamine (BDE = 86.4 kcal/mol) (Hanworth et al., 2012a), respectively at G3 and CBS-QB3 level, chain transfer [Eq. (11)] should be slightly endothermic, hence feasible in the presence of biomimetic concentrations of thiols (the rate constants of alkylamyl radical with t-BuSH and PhSH are 5 x 10⁶ and 1 x 10⁷ M⁻¹ s⁻¹ 25 °C, respectively (Musat et al., 1996)). In a study by Montevecchi and Navacchia (1998), the rate of H-atom abstraction from ArNH₂ by Ph⁺ radical was found to be similar to that of thiyl addition to an aryalkyne group, which, in turn, has been determined as 7.9 x 10⁶ M⁻¹ s⁻¹ by Ito et al. (1982). Based on the thermochemistry of equilibrium (11) it can, therefore, be estimated that the rate constant for reaction (11) is k₁₁ = 1.7 x 10⁷ M⁻¹ s⁻¹.

**Table 1** shows the effect of HOCH₂CH₂SH and Ph₂NH on peroxidation and isomerization processes of LH. When the thiol was added to the system the decrease of LOOH observed in air-equilibrated solutions indicated a lower degree of lipid peroxidation accompanied with the relevant formation of trans isomers of LH, thereby confirming the double-face behavior of thiols, which are able to protect lipids from oxidation but cause radical damage (thiyl radical-mediated isomerization). The amount of irradiation-induced lipid peroxidation at 100 Gy in control solution was at low dose rate 13% higher than at the higher dose rate. In solutions with addition of diphenylamine alone the LOOH concentration was about 60% higher at the 2.4 Gy min⁻¹ than at 274.8 Gy min⁻¹. With addition of thiol the LOOH concentration was double at the low dose rate relative to that obtained at the higher dose rate. Furthermore, irradiation of the solutions with both diphenylamine and thiol at the lower dose rate promoted three times higher LOOH concentrations than those determined at the higher dose rate. Generally, lower dose rates were found to be more efficient in LOOH formation in these systems, indicating the validity of the inverse square-root relationship with dose rate expected in homogeneous systems (Metwally and Moore, 1987; AlSheikhly and Simic, 1989; Katunin-Razem and Razem, 2000).

Ph₂NH alone inhibited the oxidation confirming the antioxidant role of aromatic amine. However, in the presence of amine there was no evidence for cis-trans isomerization, indicating that diphenylamyl radicals produced by reactions (8) and (9) do not induce LH isomerization. However, under anaerobic, and particularly under aerobic conditions, when both Ph₂NH and HOCH₂CH₂SH were present in the system, cis-trans isomerization was more marked than in the presence of the thiol alone. Furthermore, only at the lower irradiation dose rate, in the presence of both amine and thiol, LOOH formation was enhanced indicating a prooxidant role of the Ph₂NH/HOCH₂CH₂SH couple. One possible explanation for the observed loss of protective activity of aromatic amines towards the oxidation/isomerization of LH in the presence of HOCH₂CH₂SH is the occurrence of chain transfer/propagation reactions caused by amyl radical [Eq. (11)]. Based on the calculated gas phase S–H Bond Dissociation Enthalpy (BDE) for thiols (e.g. cysteine BDE = 87.3 kcal/mol) (Roux et al., 2010), and diphenylamine (BDE = 86.4 kcal/mol) (Hanworth et al., 2012a), respectively at G3 and CBS-QB3 level, chain transfer [Eq. (11)] should be slightly endothermic, hence feasible in the presence of biomimetic concentrations of thiols (the rate constants of alkylamyl radical with t-BuSH and PhSH are 5 x 10⁶ and 1 x 10⁷ M⁻¹ s⁻¹ 25 °C, respectively (Musat et al., 1996)). In a study by Montevecchi and Navacchia (1998), the rate of H-atom abstraction from ArNH₂ by Ph⁺ radical was found to be similar to that of thiyl addition to an aryalkyne group, which, in turn, has been determined as 7.9 x 10⁶ M⁻¹ s⁻¹ by Ito et al. (1982). Based on the thermochemistry of equilibrium (11) it can, therefore, be estimated that the rate constant for reaction (11) is k₁₁ = 1.7 x 10⁷ M⁻¹ s⁻¹.

**From Table 1** it can be seen that isomerization is much more effective at the low dose rate. The level of trans isomers of LH in N₂O-saturated solutions was much higher than under air-equilibrated conditions, when thiol was present, either alone or with amine. As it was expected, in N₂O-saturated solutions LOOH formation in these systems, indicating the validity of the inverse square-root relationship with dose rate expected in homogeneous systems (Metwally and Moore, 1987; AlSheikhly and Simic, 1989; Katunin-Razem and Razem, 2000).
concentrations were below detection limit.

In the absence of literature in this regard we wondered whether the diphenylnitroxyl radicals Ph2NO+ which can be formed from the corresponding Ph2N according to Eq. (10), could contribute to the enhanced isomerization of LH. In order to clarify whether and to which extent Ph2NO+ radicals are involved in cis–trans isomerization we turned to EPR spectroscopy and product studies. The results allowed excluding that under our experimental settings Ph2NO+ or in general persistent nitroxyl radicals can induce significant cis–trans isomerization (see Supporting Information for full details). The mechanism for the cis–trans isomerization in the presence of HOCH2CH2SH and Ph2NH is summarized in Scheme 2.

3.2. Irradiation of linoleic acid in micelles

Irradiated N2O-saturated samples in the presence or absence of thiol/amine did not show any formation of LOOH. Fig. 1 shows dose-profiles of LOOH formation under air-equilibrated conditions. LOOH is produced in the presence (+) or absence of thiol (●). However, LOOH was negligible when Ph2NH was added, either alone (●) or with HOCH2CH2SH before irradiation (◆), showing a good protective activity from lipid peroxidation in micelles, despite the lower concentration as compared to experiments in homogeneous solution.

The same samples were analysed for geometrical isomers distribution during lipid peroxidation. Fig. 2 (A) displays the development of geometrical isomers as a function of dose under N2O-saturated conditions in the presence of HOCH2CH2SH (open symbols) or in the presence of HOCH2CH2SH/Ph2NH (solid symbols). In both cases the results show the disappearance of 9c,12c–C18:2 (◆,□) being replaced by the formation of mono-trans isomers (●,▲) and 9t,12t–C18:2 (◆,□).

As expected, isomerization was more marked in the absence of oxygen, however under air-equilibrated conditions the cis–trans isomerization still operates. The disappearance of 9c,12c–C18:2 (●) matches well with the formation of mono-trans isomers (●,▲) and 9t,12t–C18:2 (◆,□), under the conditions where the LOOH formation was detectable (cf. Fig. 1).

Results obtained in this part of work are summarized in Table 2. As compared with the results obtained in homogeneous LH solutions, in LH micelles the Ph2NH/thiol couple inhibited peroxidation processes with modest effect on isomerization level. Low concentration of Ph2NH (83 μM) slightly inhibited isomerization when the thiol is present in aerobic and anaerobic conditions. This effect can be explained by quenching of thiol radicals by Ph2NH [Eq. (11)].

However, at variance with what was observed in homogeneous solution, Ph2NH had negligible effect on thyl radical-mediated isomerization in the absence of oxygen. This result can, in part, be explained by the lower concentration of Ph2NH as compared to homogenous-phase experiments; however, it could also depend on different interactions of aminyl radicals and thiol due to partition effects in the heterogeneous environment.

In comparison with the results of lipid transformation processes investigated as function of dose rate in homogeneous solutions, LOOH concentrations formed at the same dose and at the

### Table 1

The amounts of LOOH of air-equilibrated [EtOH:H2O]=(1:1) solutions (third column) and trans LH isomers of air-equilibrated or N2O-saturated [EtOH:H2O]=(1:1) solutions generated by gamma irradiation with 100 Gy as function of dose rate; 0.5 mM LH, 0.28 mM TWEEN-20, 5 mM PB, pH 5.

<table>
<thead>
<tr>
<th>Dose rate/Gy min⁻¹</th>
<th>Additives</th>
<th>[LOOH]/10⁻⁶ M</th>
<th>% Isomers LH 9c,12c/9c,12t/9t,12c/9t,12t*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Air</td>
<td>N2O</td>
</tr>
<tr>
<td>2.4</td>
<td>None (control)</td>
<td>27.5 ± 0.4</td>
<td>100/0/0/0</td>
</tr>
<tr>
<td></td>
<td>2.8 mM HOCH2CH2SH</td>
<td>18.8 ± 0.7</td>
<td>90/4.7/4.8/0</td>
</tr>
<tr>
<td></td>
<td>0.5 mM Ph2NH</td>
<td>23.8 ± 0.5</td>
<td>100/0/0/0</td>
</tr>
<tr>
<td></td>
<td>2.8 mM HOCH2CH2SH and 0.5 mM Ph2NH</td>
<td>36.1 ± 1.2</td>
<td>69.4/13.7/11.5/3.5</td>
</tr>
<tr>
<td>274.8</td>
<td>None (control)</td>
<td>24.3 ± 3.2</td>
<td>100/0/0/0</td>
</tr>
<tr>
<td></td>
<td>2.8 mM HOCH2CH2SH</td>
<td>9.3 ± 0.4</td>
<td>95.2/2.2/2.2/0.4</td>
</tr>
<tr>
<td></td>
<td>0.5 mM Ph2NH</td>
<td>14.9 ± 0.5</td>
<td>100/0/0/0</td>
</tr>
<tr>
<td></td>
<td>2.8 mM HOCH2CH2SH and 0.5 mM Ph2NH</td>
<td>12.9 ± 1.8</td>
<td>93.2/3.3/3.1/0.4</td>
</tr>
</tbody>
</table>

* Reported values represent the mean of three independent measurements (p < 0.05); errors are less than ± 5%.

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**Scheme 2.** Role of aminyl radicals in promoting cis–trans isomerization in the presence of O2.
In the presence of 2.8 mM HOCH$_2$CH$_2$SH, open symbols (○ ○ ○), and the formation of 9t,12C–C18:2 (● ● ●), and the formation of 9t,12C–C18:2 (□ □ □); experiments were performed either in the presence of 2.8 mM HOCH$_2$CH$_2$SH, open symbols (○ ○ ○), or in the presence of 2.8 mM HOCH$_2$CH$_2$SH and 83 μM Ph$_2$NH, solid symbols (● ● ●).

we repeated the experiments in LH micelles using α-tocopherol (ArOH) as one of the most efficient and biologically relevant radical scavengers (Valgimigli and Pratt, 2012).

The amounts of LOOH under aerobic conditions (third column) and trans isomers of LH generated by gamma irradiation with 100 Gy of LH micelles in aerobic and anaerobic conditions as function of dose rate; 0.5 mM LH, 0.28 mM Tween-20*, 5 mM PB, pH 5.

<table>
<thead>
<tr>
<th>Dose rate/ Gy min$^{-1}$</th>
<th>Additives</th>
<th>[LOOH]/10$^{-6}$ M</th>
<th>% Isomers of LH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Air</td>
</tr>
<tr>
<td>2.4</td>
<td>None (control)</td>
<td>76.7 ± 3.1</td>
<td>100/0/0/0</td>
</tr>
<tr>
<td>2.8 mM HOCH$_2$CH$_2$SH</td>
<td>7.0 ± 0.2</td>
<td>88.3/5.4/0/0</td>
<td>112/20.8/8</td>
</tr>
<tr>
<td>0.5 mM Ph$_2$NH</td>
<td>18.5 ± 0.9</td>
<td>100/0/0/0</td>
<td>100/0/0/0</td>
</tr>
<tr>
<td>2.8 mM HOCH$_2$CH$_2$SH</td>
<td>17.9 ± 0.9</td>
<td>94.3/3.1/1/20</td>
<td>17.7/24.2/20</td>
</tr>
<tr>
<td>and 83 μM Ph$_2$NH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>274.8</td>
<td>None (control)</td>
<td>14.4 ± 0.4</td>
<td>100/0/0/0</td>
</tr>
<tr>
<td>2.8 mM HOCH$_2$CH$_2$SH</td>
<td>29.0 ± 0.9</td>
<td>96.4/2.0/1.6/0</td>
<td>46.5/21.7/16/2</td>
</tr>
<tr>
<td>0.5 mM Ph$_2$NH</td>
<td>5.5 ± 0.1</td>
<td>100/0/0/0</td>
<td>100/0/0/0</td>
</tr>
<tr>
<td>2.8 mM HOCH$_2$CH$_2$SH</td>
<td>6.3 ± 0.2</td>
<td>96.3/19.5/1.9/0</td>
<td>52.9/19.6/17.6</td>
</tr>
<tr>
<td>and 83 μM Ph$_2$NH</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Reported values represent the mean of three independent measurements ($p < 0.05$); errors are less than ± 5%.

Table 2

Fig. 2. The geometrical isomers distribution of LH as function of irradiation dose (dose rate 274.8 Gy min$^{-1}$) in micelles (0.5 mM LH, 0.28 mM Tween-20*, 5 mM PB at pH 5) under N$_2$O-saturated (A) or air-equilibrated (B) conditions; the disappearance of 9t,12C–C18:2 (● ● ●), the formation of 9t,12C–C18:2 + 9c,12t–C18:2 (○ ○ ○), and the formation of 9t,12t–C18:2 (□ □ □); experiments were performed either in the presence of 2.8 mM HOCH$_2$CH$_2$SH, open symbols (○ ○ ○), or in the presence of 2.8 mM HOCH$_2$CH$_2$SH and 83 μM Ph$_2$NH, solid symbols (● ● ●).

In order to compare the activity of Ph$_2$NH in radiation induced peroxidation and thyl radical-catalyzed cis–trans isomerization dose rate of 2.4 Gy min$^{-1}$ in control solution and in solutions with added diphenyl amine were 80% and 70%, respectively, higher than those obtained at 274.8 Gy min$^{-1}$. However, the presence of both diphenylamine and thyl at the lower dose rate promoted 65% higher LOOH concentrations than those determined at the highest dose rate. With the exception for the case when thyl alone was added to control micellar solution, the lower dose rate was found to be more efficient in LOOH formation. Although no inverse square-root correlation with dose rate was originally tested in those systems, this dose rate dependence of peroxidation found in our model system indicates that an inverse square root relation between the radiation-chemical yield of LOOH and dose rate in homogeneous medium may also hold in our micellar model system.

In order to compare the activity of Ph$_2$NH in radiation induced peroxidation and thyl radical-catalyzed cis–trans isomerization we repeated the experiments in LH micelles using α-tocopherol (ArOH) as one of the most efficient and biologically relevant radical scavengers (Valgimigli and Pratt, 2012).

In the presence of α-tocopherol the alkyl radical R$^*$ generates the aryloxyl radical from α-tocopherol [Eq. (14)], $k_{14} = 6.0 \times 10^6$ M$^{-1}$ s$^{-1}$ at 25 °C (Franchi et al., 1999).

$R^* + ArOH \rightarrow Rh + ArO^*$

(14)

$ROO^* + ArOH \rightarrow ROOH + ArO^*$

(15)

$HOCH_2CH_2S^+ + ArOH \rightarrow HOCH_2CH_2SH + ArO^*$

(16)

In O$_2$-atmosphere peroxyl radicals also give aryloxyl radicals from α-tocopherol [Eqs. (6) and (15)], $k_{15} = 3.2 \times 10^6$ M$^{-1}$ s$^{-1}$ (Valgimigli and Pratt, 2012). Fig. 3 shows the formation of LOOH as function of dose in LH micelles. The antioxidant activity of α-tocopherol is confirmed by a significant decrease of LOOH formation when dose increases in the presence of α-tocopherol. Protection is even higher upon combination with the thyl. While isomerization was induced in the presence of thiol alone (○ ○ ○ Fig. 4), under aerobic, and especially under anaerobic conditions, this process was significantly inhibited upon addition of 50 μM α-tocopherol. The good protection of α-tocopherol against lipid peroxidation/isomerization in the presence of thyl can be explained based on the available kinetic information for reaction (16). The reaction of thyl radicals (e.g. from cysteine) with tocopherol’s analog Trolox is known to occur by hydrogen abstraction [Eq. (16)] with $k_{16} = 1 \times 10^6$ M$^{-1}$ s$^{-1}$, whereas for the reverse reaction only a limiting value ($k_{16} < 10^3$ M$^{-1}$ s$^{-1}$) is available (Davies et al., 1988; De Koning 2002).

Therefore, the presence of biologically significant concentrations of α-tocopherol (50 μM) inhibited lipid peroxidation with simultaneous inhibition of thyl-induced trans isomerization of LH (Fig. 4). The inhibition of isomerization by α-tocopherol was more effective than what was observed with even higher concentration of the aromatic amine (Fig. 2A).
aromatic amines from physiological antioxidants like α-tocopherol, despite the ability of both antioxidants to protect against peroxidation. Free radical chemistry described in this work is expectedly relevant in biological environments, where thiols are the most abundant endogenous antioxidants (at mM levels) and di-phenylamine might be introduced with the diet as food preservative. The arylamyl radical reactivity bringing to overgeneration of thyl radicals stimulates further investigation of a novel process of toxicity of secondary aromatic amines.

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**Appendix A. Supplementary material**

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.radphyschem.2015.11.018.

**References**


