cis−*trans* Isomerization of Monounsaturated Fatty Acid Residues in Phospholipids by Thiyl Radicals

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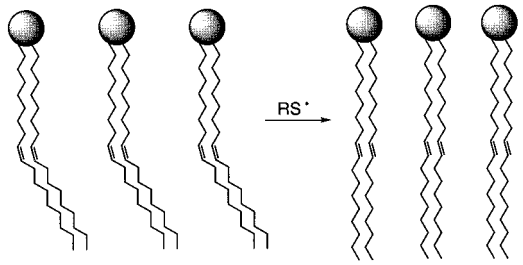
Abstract: Thiyl radicals reversibly attack the double bonds of methyl oleate and dioleoyl phosphatidyl choline (DOPC), thus producing methyl elaidate and the corresponding phospholipids containing *trans*-fatty acid residues in high yield. These processes are radical chain reactions with relatively long chain lengths. The rate constant for the β-elimination of a thiyl radical from the adduct radical has been estimated to be 6×10^6 s^{-1} at ambient temperature. The *cis*−*trans* isomerization of fatty acid residues in DOPC vesicles (multilamellar vesicles and large unilamellar vesicles made by the extrusion technique) by a thiyl radical, generated from biologically relevant thiols, has also been studied in detail. The presence of 0.2 mM oxygen does not influence the effectiveness of *cis*−*trans* isomerization in both homogeneous solution and lipid vesicles. This process, which does not cause lipid degradation but permanent modification of the membrane constituents, ultimately influences the barrier properties and functions of biological membranes.

Introduction

In cell membranes, the *cis* configuration of unsaturated fatty acid residues regulates the self-organization of phospholipids. The *cis*−*trans* isomerization, caused by a chemical agent (catalytic hydrogenation of lipids[1]) or an enzyme (mitochondrial isomerases[2]), can affect the lipid assembly since the *trans* arrangement resembles the structure of saturated fatty acids (cf. Scheme 1). Membranes made of *trans*-fatty acids can form a more rigid packing of phospholipids than membranes containing *cis*-fatty acids, and therefore the physical properties (e.g., microviscosity or thermal phase behavior) of the bilayer are affected.

The importance of the double bond arrangement on the membrane properties has been assessed in a number of studies. Changes in the morphology of mitochondrial membranes[3] and in the thermotropic behavior of membrane lipids,[4] obtained by yeast auxotrophs which were fed with *trans*-unsaturated fatty acids, have indeed been observed. The equivalence between a *trans* and a saturated fatty acid was confirmed by steady-state fluorescence polarization studies of vesicles composed of phospholipids from the liver mitochondria of rats fed with elaidic acid.[5] The biological significance of *trans*-unsaturated fatty acids

Scheme 1



was proven in the case of some bacteria which utilize the enzymatic *cis*−*trans* isomerization as a strategy for protecting themselves from increases in ambient temperature[6,7] or from the toxicity of compounds such as phenols.[8] The interaction between proteins and lipids in membranes has also been found to be dramatically influenced by the geometrical isomers of the fatty acid chains.[9] It is worth mentioning that the effect on humans of *trans*-unsaturated fatty acids supplied by the diet is a recently growing concern in nutrition.[10]

Radical-based damage of biologically relevant molecules has increasingly attracted the interest of researchers from different scientific fields, from chemistry to medicine. To the best of our knowledge, radical processes involving membrane phospholipids

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(9) Helmkamp, G. M., Jr. *Biochemistry* **1980**, *19*, 2050−2056.

(10) (a) Ratnayake, W. M. N.; Chen, Z.-Y. *Lipids* **1996**, *31* (Suppl.), 279−282 and references therein. (b) For a review, see: Katan, M. B.; Zock, P. L.; Mensink, R. P. *Annu. Rev. Nutr.* **1995**, *15*, 473−493.

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are limited to polyunsaturated fatty acid (PUFA) peroxidation,[11,12] and to a recently reported homolytical cleavage of lysophospholipids.[13]

From a chemical perspective, the *cis* to *trans* conversion of double bonds is a thermodynamic favored process,[14] which can also occur by the reversible addition of a free radical. During our study on the isomerization of double bonds caused by radical species,[15] we found that thiyl radicals are among the most efficient isomerizing agents.[16] Since then, we have been attracted by the idea of a similar reaction taking place in the case of naturally occurring unsaturated phospholipids containing *cis*-residues. This event should change the double bond geometry, thus leading to the thermodynamically more stable *trans*-isomer. We report herein that thiyl radicals are indeed able to induce the isomerization of *cis*-monounsaturated phospholipids (Scheme 1).[20,21] This process was observed using lipid solutions and liposome vesicles under anoxic or aerobic conditions. We showed that phospholipids containing *trans*-fatty acid residues are produced in high yield.

Results and Discussion

Generation of Radicals. Selectively generated thiyl radicals were produced by the reaction of an alkyl radical with the corresponding thiol. Alkyl radicals were generated by either thermal decomposition of azo derivatives or γ-irradiations. The choice of thiol was based on the experimental conditions. In a homogeneous system where *t*-BuOH was used as a solvent, benzenethiol (PhSH) and β-mercaptoethanol ($HOCH_2CH_2SH$) were chosen. In a heterogeneous system (vesicles), thiols can be either incorporated into the bilayer, or dissolved in the aqueous phase. Amphiphilic $HOCH_2CH_2SH$ was used without any concern about the partition of thiol between hydrophobic and hydrophilic regions.[25] However, two other biologically related thiols of different lipophilicity such as glutathione (GSH) and cysteine (CySH) were also used.[25]

The lipophilic initiators azobis(isobutyronitrile) (AIBN) and azobis(dimethylvaleronitrile) (AMVN) and the hydrophilic azobis(2-amidinopropane) hydrochloride (AAPH) were used at 71, 54, and 37 °C, respectively, taking into account their half-life times (eq 1).

$$R{-}N{=}N{-}R \xrightarrow{\Delta} N_2 + 2\,R^{\bullet} \qquad (1)$$

Radiolysis of neutral water leads to the species e_{aq}^-, $HO^{\bullet}$, and $H^{\bullet}$ as shown in eq 2, where the values in parentheses represent the yields expressed in terms of *G*-values (μmol J^{-1}).[26]

$$H_2O \rightsquigarrow e_{aq}^- (0.27),\ HO^{\bullet} (0.28),\ H^{\bullet} (0.06) \qquad (2)$$

Thiyl radicals were generated by irradiating N_2O-saturated solutions containing *i*-PrOH at natural pH. The presence of N_2O efficiently transforms e_{aq}^- into the $HO^{\bullet}$ radical (eq 3, $k_3 = 9.1 \times 10^9$ M^{-1} s^{-1}). Hydrogen abstraction from *i*-PrOH by $HO^{\bullet}$ and $H^{\bullet}$ produces $(CH_3)_2{}^{\bullet}COH$ (eqs 4 and 5, $k_4 = 1.9 \times 10^9$ M^{-1} s^{-1}, $k_5 = 7.4 \times 10^7$ M^{-1} s^{-1}). The $(CH_3)_2{}^{\bullet}COH$ in turn reacts with the thiol to give the corresponding thiyl radical (eq 6, $k_6 \cong 2 \times 10^8$ M^{-1} s^{-1}).[26,27]

$$e_{aq}^- + N_2O \xrightarrow{H_2O} N_2 + HO^{\bullet} + HO^- \qquad (3)$$

$$HO^{\bullet} + (CH_3)_2CHOH \longrightarrow (CH_3)_2\dot{C}OH + H_2O \qquad (4)$$

$$H^{\bullet} + (CH_3)_2CHOH \longrightarrow (CH_3)_2\dot{C}OH + H_2 \qquad (5)$$

$$(CH_3)_2\dot{C}OH + RSH \longrightarrow (CH_3)_2CHOH + RS^{\bullet} \qquad (6)$$

Radiolysis of *t*-BuOH leads to the species e_{sol}^-, and $^{\bullet}CH_2C(CH_3)_2OH$ as shown in eq 7. In N_2O-saturated solutions e_{sol}^- is transformed into the $HO^{\bullet}$ radical (eq 8). Hydrogen abstraction from *t*-BuOH by $HO^{\bullet}$ produces $^{\bullet}CH_2C(CH_3)_2OH$ (eq 9, $k_9 = 6.0 \times 10^8$ M^{-1} s^{-1}) and therefore, we can consider the $G[^{\bullet}CH_2C(CH_3)_2OH]$ to be ca. 0.65 μmol J^{-1}.[28] It is known that in water the $^{\bullet}CH_2C(CH_3)_2OH$ abstracts hydrogen from the $HOCH_2CH_2SH$ with a rate constant of $k_{10} = 8.2 \times 10^7$ M^{-1} s^{-1} to give the corresponding thiyl radical (eq 10).[26,27] The rates of thiol trapping of alkyl radicals are solvent dependent.[30] The

(11) For some representative reviews, see: Porter, N. A. *Acc. Chem. Res.* **1986**, *19*, 262−268. Niki, E. In *Organic Peroxides*; Ando, W., Ed.; Wiley: New York; 1992; pp 764−787. Barclay, L. C. R. *Can. J. Chem.* **1993**, *33*, 1−16.

(12) For the autoxidation of methyl oleate, see: Porter, N. A.; Mills, K. A.; Carter, R. L. *J. Am. Chem. Soc.* **1994**, *116*, 6690−6696.

(13) Muller, S. N.; Batra, R.; Senn, M.; Giese, B.; Kisel M.; Shadyro, O. *J. Am. Chem. Soc.* **1997**, *119*, 2795−2803.

(14) For example, see: Sonnet, P. E. *Tetrahedron* **1980**, *36* 557−604. Saltiel, J.; Sears, D. F., Jr.; Ko, D.-H.; Park, K.-M. In *CRC Handbook of Organic Photochemistry and Photobiology*; Horspool, W. H., Song, P. S., Eds.; CRC Press: Boca Raton, 1995; Chapter 1.

(15) Chatgilialoglu, C.; Ballestri, M.; Ferreri, C.; Vecchi, D. *J. Org. Chem.* **1995**, *60*, 3826−3831.

(16) The ability of thiyl radicals to isomerize double bonds was first reported 40 years ago[17] and has since attracted considerable attention particularly in organic synthesis.[18] On the other hand, thiols and the corresponding thiyl radicals are of considerable importance in biological processes, where they can act as repairing and as damaging agents, respectively.[19]

(17) (a) Pallen, R. H.; Sivertz, C. *Can. J. Chem.* **1957**, *35*, 723−733. (b) Walling, C.; Helmreich, W. *J. Am. Chem. Soc.* **1959**, *81*, 1144−1148. (c) For a review of early work, see: Kice, J. L. In *Free Radicals*; Kochi, J. K., Ed.; Wiley: New York, 1973; Vol. 2, pp 711−740.

(18) For recent reviews, see: (a) Chatgilialoglu, C.; Guerra, M. In *Supplement S: The Chemistry of Sulfur-containing Functional Groups*; Patai, S., Rappoport, Z., Eds.; Wiley: London, 1993; Chapter 8, pp 363−394. (b) Chatgilialoglu, C.; Bertrand, M. P.; Ferreri, C. In *S-Centered Radicals*; Alfassi, Z. B., Ed.; Wiley: Chichester, 1999; pp 312−354.

(19) *Sulfur-centered Reactive Intermediates in Chemistry and Biology*; Chatgilialoglu, C., Asmus, K.-D., Eds.; Plenum Press: New York, 1990.

(20) For a preliminary communication, see: Ferreri, C.; Costantino, C.; Landi, L.; Mulazzani, Q. G.; Chatgilialoglu, C. *Chem. Commun.* **1999**, 407−408. In this communication, Fig 2b refers to MLVs with 75 mM of $HOCH_2CH_2SH$ and not to LUVETs with 7 mM of $HOCH_2CH_2SH$, as euroneously reported.

(21) It has been reported that oleic acid shows no measurable reaction with alkyl thiyl radicals by pulse radiolysis techniques, and consequently, an addition of $RS^{\bullet}$ to the double bonds seems to be of minor if any importance.[22] Recently *cis*−*trans* isomerization of polyunsaturated fatty acid residues in phospholipids by free radicals has also been reported. Schwinn et al. proposed that *cis*−*trans* isomerization in linoleate moieties is the fate of the pentadienyl radical which reacts with thiols in different conformations.[23] On the other hand, Balazy and co-workers proposed a reversible addition of $^{\bullet}NO_2$ radicals to arachidonic moieties.[24]

(22) Schöneich, C.; Bonifacic, M.; Dillinger, U.; Asmus, K.-D. In *Sulfur-Centered Reactive Intermediates in Chemistry and Biology*; Chatgilialoglu, C., Asmus, K.-D., Eds.; Plenum Press: New York, 1990; pp 367−376.

(23) Schwinn, J.; Sprinz, H.; Dröβler, K.; Leistner, S.; Brede, O. *Int. J. Radiat. Biol.* **1998**, *74*, 359−365.

(24) Jiang, H.; Kruger, N.; Lahiri, D. R.; Wang, D.; Vatèle, J.-M.; Balazy, M. *J. Biol. Chem.* **1999**, *274*, 16235−16241.

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(26) Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1988**, *17*, 513 and references therein.

(27) Ross, A. B., Mallard, W. G.; Helman, W. P.; Buxton, G. V.; Huie, R. E.; Neta, P. *NDRL-NIST Solution Kinetic Database*−*Ver. 3*; Notre Dame Radiation Laboratory: Notre Dame, IN; NIST Standard Reference Data: Gaithersburg, MD, 1998.

(28) On the basis of the total $G = 0.61$ μmol J^{-1} in water (eq 2) and $G(^{\bullet}CH_2OH) = 0.67$ μmol J^{-1} in methanol,[29] the assumption of $G[^{\bullet}CH_2C(CH_3)_2OH] = 0.65$ μmol J^{-1} seems reasonable.

(29) Spinks, J. W. T.; Woods, R. J. *An Introduction to Radiation Chemistry*, 3rd ed.; Wiley: New York, 1990; p 421.

(30) Tronche, C.; Martinez, F. N.; Horner, J. H.; Newcomb, M.; Senn, M.; Giese, B. *Tetrahedron Lett.* **1996**, *33*, 5845−5848.

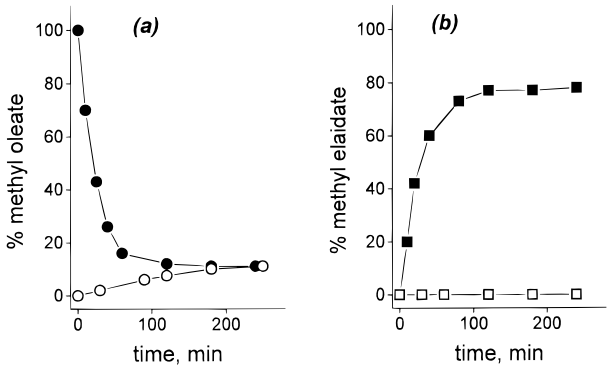


Figure 1. (a) Time profiles of methyl oleate in *t*-BuOH at 71 °C: (●) 0.15 M methyl oleate with 75 mM PhSH and 30 mM AIBN; (○) 0.15 M methyl elaidate with 75 mM PhSH and 30 mM AIBN. (b) Time profiles of methyl elaidate in *t*-BuOH at 54 °C: (■) DOPC with 75 mM $HOCH_2CH_2SH$ and 30 mM AMVN; (□) DOPC without thiol and 30 mM AMVN.

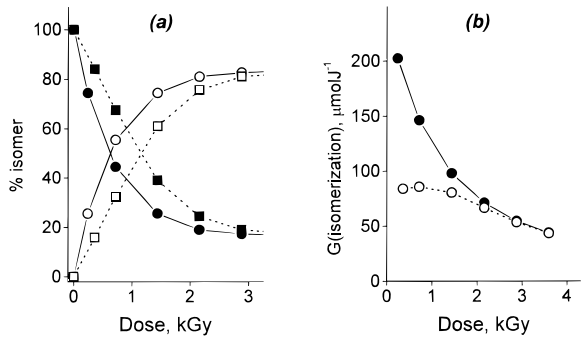
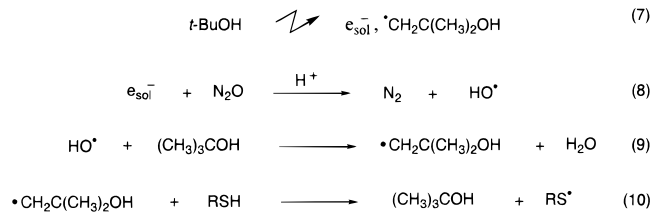


Figure 2. (a) Methyl oleate (●, ■) or methyl elaidate (○, □) vs dose from the γ-radiolysis (23.3 Gy min^{-1}) of methyl oleate in N_2O-saturated *t*-BuOH at 22 °C: (●, ○) without oxygen; (■, □) with 2.34×10^{-4} M oxygen. (b) *G*(isomerization) vs dose: (●) without oxygen; (○) with oxygen.

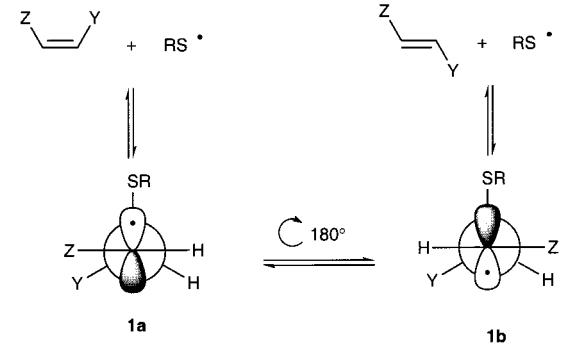
rate constant for the reaction of a primary alkyl radical with octanethiol is measured to be 1.9×10^7 M^{-1} s^{-1} at 30 °C in THF.[30]



Isomerization of Methyl Oleate. A solution of 0.15 M methyl oleate, 0.075 M PhSH, and 0.03 M AIBN in *t*-BuOH was heated at 71 °C for 4 h. The reaction was monitored by GC, and its profile is shown in Figure 1a. By replacing the methyl oleate with methyl elaidate, the same *cis/trans* ratio, i.e., 11/89, was reached in 4 h. These results are in good agreement with the early work of Moussebois and Dale which showed that *cis*- and *trans*-2-butene are isomerized by the PhS• radical.[31] For comparison, we studied the isomerization of methyl oleate (0.15 M) with $HOCH_2CH_2SH$ (0.075 M) in *t*-BuOH using either AMVN (0.03 M) at 54 °C or γ-irradiation of a N_2O-saturated solution at 22 °C. Similar results were obtained in the two experiments. Figure 2a shows the irradiation dose profiles of the disappearance of methyl oleate (solid circles) and the formation of methyl elaidate (open circles). The percentage of

(31) Moussebois, C.; Dale, J. *J. Chem. Soc.* **1966**, 260−264, 264−267.

Scheme 2



the isomeric composition after completion was *cis/trans* = 17/83. It is worth pointing out that the conversion of the starting material to the equilibrium mixture was quantitative in all the experiments (yields >95% using methyl palmitate as an internal standard). The product yield (mol kg^{-1}) divided by the absorbed dose (1 Gy = 1 J kg^{-1}) gives the radiation chemical yield or *G*(isomerization). Figure 2b (solid circles) shows the plot of *G*(isomerization) vs dose. The extrapolation of the last two points to zero dose gives $G \cong 230$ μmol J^{-1}. Assuming that the *G*(RS•) is 0.65 μmol J^{-1} (eqs 9 and 10), we calculated the chain length to be 350 at the initial phase.

The mechanism that we conceived for this transformation includes hydrogen abstraction from the thiol, the addition of thiyl radicals to the *cis* double bond of methyl oleate to give **1a**, half-rotation about the carbon−carbon bond of the radical intermediate to give **1b**, and ejection of the thiyl radical by β-scission (Scheme 2).[15]

Figure 2a (squares) shows the dose profile of the isomerization from a solution saturated by 10% oxygen. In comparison with the deoxygenated solution experiment (circles) the reaction reaches the same equilibrium mixture without loss of starting material but with a slightly longer reaction time. Figure 2b (open circles) shows the *G*(isomerization) vs dose which gives a $G \cong 85$ μmol J^{-1} by extrapolation of the last two points to zero dose. The decrease of *G*(isomerization) from 230 to 85 μmol J^{-1} in the presence of 2.34×10^{-4} M oxygen[32] is easily explained by assuming a smaller concentration of thiyl radicals. In fact, in the presence of 0.075 M thiol and 2.34×10^{-4} M oxygen, reaction 10 will be only 3 times faster than reaction 11 since k_{10} and k_{11} are ca. 2×10^7 and 2×10^9 M^{-1} s^{-1}, respectively.[34] In other words, the effectiveness of the *cis*−*trans* isomerization with or without oxygen is the same.

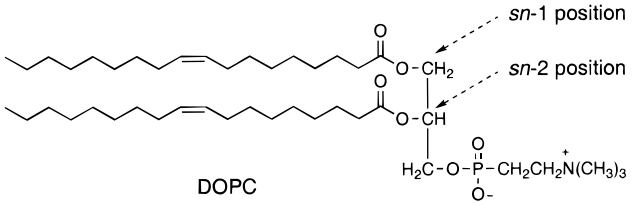
Furthermore, assuming a rate constant of 2×10^9 M^{-1} s^{-1} for the reaction of radical **1** with oxygen (cf. Scheme 2), we calculated a pseudo-first-order rate constant of 4.7×10^5 s^{-1} for the oxygen trapping.[32] The above results suggest that the β-elimination of RS• radical (Scheme 2) must be at least 1 order

(32) The solubility of molecular oxygen in *t*-BuOH is determined to be 2.34×10^{-3} M at rt.[33] Therefore, 10% oxygen-saturated solution is equal to 2.34×10^{-4} M.

(33) Cipollone, M.; di Palma, C.; Pedulli, G. F. *Appl. Magn. Reson.* **1992**, *3*, 98−102.

(34) The k_{11} has been measured in water; see: Mark, G.; Schuchmann, M. N.; Schuchmann, H. P.; von Sonntag, C. J. *Photochem. Photobiol., A* **1990**, *55*, 157−168. von Piechowski, M.; Thelen, M. A.; Hoigne, J.; Buehler, R. E. *Ber. Bunsen-Ges. Phys. Chem.* **1992**, *96*, 1448−1454. However, the rate constants for the reaction of an alkyl radical with oxygen are similar in organic solvents (cf. Maillard, B.; Ingold, K. U.; Scaiano, J. C. *J. Am. Chem. Soc.* **1983**, *105*, 5095−5099).

Scheme 3

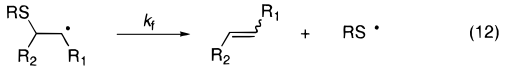


of magnitude higher than 4.7×10^5 s^{-1}, i.e., the trapping of radical **1** by oxygen.

Isomerization of Dioleoyl Phosphatidyl Choline (DOPC) in *t*-BuOH. A set of experiments similar to those with methyl oleate was carried out with a *t*-BuOH solution of DOPC (Scheme 3) since it is known that phospholipids do not aggregate in this solvent.[35] A DOPC/chloroform solution (3 mL; 0.15 mmol of oleate contents) was evaporated in a test tube under an argon stream. A thin film formed which was kept under vacuum for 30 min. *t*-BuOH (1 mL), $HOCH_2CH_2SH$ (0.075 mmol), and AMVN (0.030 mmol) were added, and the solution was degassed with argon. The reaction mixture was warmed at 54 °C, and aliquots (100 μL) were processed at different times. Alternatively, *t*-BuOH (1 mL) and $HOCH_2CH_2SH$ (0.007 mmol) were added, and the solution was divided into aliquots of 100 μL in different tubes followed by saturation with N_2O prior to γ-irradiation. After transesterification[36] of the phospholipid at different reaction times, the methyl oleate/methyl elaidate ratios were obtained by GC analysis. Figure 1b (solid squares) shows the time profiles of methyl elaidate (i.e., *trans*-isomer) formation as well as the absence of isomerization without the thiol (open squares). Also in these experiments, the conversion of the starting material to the equilibrium mixture was quantitative.

The percentages of the isomeric composition of methyl oleate/methyl elaidate after completion vary slightly with temperature. At 71, 54, and 22 °C the ratios are 22/78, 20/80, and 17/83, respectively; by using the Boltzmann distribution expression, we obtained ΔE values of 0.87, 0.90, and 0.93 kcal mol^{-1}, respectively, which account for the difference in the stability of the two isomers, i.e., $\Delta_f H°(trans) - \Delta_f H°(cis) = -1.0$ kcal mol^{-1}.[37]

***β*-Elimination of RS• Radicals.** To our knowledge, quantitative measurements of the β-elimination of alkanethiyl radicals (RS•) from any carbon-centered radical of type **2** are absent from the literature (eq 12). An isolated measurement obtained



(12)

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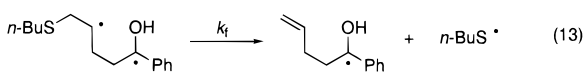
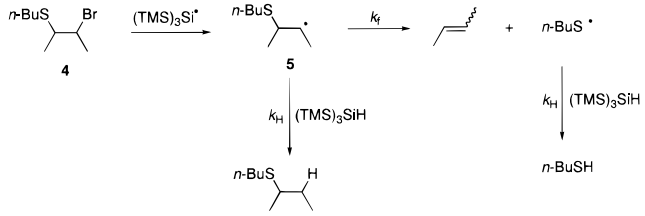


(13)

3

for the analogous biradical **3** is known for which Wagner and co-workers have reported a $k_f = 2.7 \times 10^5$ s^{-1} at 25 °C for the β-elimination of the *n*-BuS• radical (eq 13).[38] However, it is worth mentioning that the rate constant for the analogous β-elimination of the PhS• radical ranges from 1×10^6 to 1×10^8 s^{-1} depending on the stabilizing effects of the substituents R_1 and R_2 both in the intermediate alkyl radical and in the alkene product (cf. eq 12).[18,39]

Scheme 4



On the basis of the oxygen experiments described with methyl oleate the RS• elimination from radical **1** (Scheme 2) must be at least 20 times faster than the value of 2.7×10^5 s^{-1} observed for the biradical **3**. This means that the β-alkyl substituent R_2 in radical **2** should have a strong accelerating effect in the β-elimination of the RS• radical (eq 12).

An indirect procedure for measuring the rate constant of a unimolecular process involves competition between this process and a bimolecular path of the radical (*free-radical clock* methodology).[40] In his review, Newcomb summarized competition methods for this purpose.[41] We envisaged that the rate constant, k_f, of the *n*-BuS• radical β-elimination from radical **5** can be obtained by a chain reaction of bromide **4** with $(TMS)_3SiH$ (Scheme 4), providing that conditions can be found in which the radical **5** is partitioned between the two reaction channels (i.e., a reaction with the $(TMS)_3SiH$ and the β-fragmentation of the radical). Furthermore, the Arrhenius expression for the reaction of secondary alkyl radicals with $(TMS)_3SiH$ is known,[42] and strong experimental evidence exists that the reaction of the thiyl radical with $(TMS)_3SiH$ is an efficient process.[44]

This scenario can be achieved at −70 °C if the $(TMS)_3SiH$ concentration remains essentially constant during the course of the reaction (bimolecular process under pseudo-first-order conditions). Under these conditions eq 14 holds.

$$\frac{[\mathbf{6}]}{[n\text{-BuSH}]} = \frac{k_H}{k_f}[(TMS)_3SiH] \qquad (14)$$

The quantities of **6** and *n*-BuSH were obtained by GC analysis, following the photolytically initiated radical reaction, and by using an internal standard. The ratio [**6**]/[*n*-BuSH] varied in the manner expected with a change in the silane concentration.[45a] The $k_H/k_f = 0.31 \pm 0.05$ M^{-1} was obtained as the average of six different experiments.[45] Taking $k_H = 4.5 \times 10^3$ M^{-1} s^{-1} at −70 °C,[42] a k_f value was calculated to be 1.4×10^4

(35) Barclay, L. R. C.; McNeil, J. M.; VanKessel, J.; J. Forrest, B.; Porter, N. A.; Lehman, L. S.; Smith, K. J.; Ellington, J. C., Jr. *J. Am. Chem. Soc.* **1984**, *106*, 6740−6747.

(36) The transesterification in an alkaline medium is preferable, as reported by Kramer, J. F. K.; Fellner, V.; Dugan, M. E. R.; Sauer, F. D.; Mossoba M. M.; Yurawecz, M. P. *Lipids* **1997**, *32*, 1219−1228.

(37) *Handbook of Chemistry and Physics*, 74th ed.; Lide, D. R., Ed.; CRC Press: Boca Raton, 1993−94.

(38) Wagner, P. J.; Sedon, J. H.; Lindstrom, M. J. *J. Am. Chem. Soc.* **1978**, *100*, 2579−2580.

(39) Ito, O. In *S−Centered Radicals*; Alfassi, Z. B., Ed.; Wiley: Chichester, 1999; pp 193−224.

(40) Griller, D.; Ingold, K. U. *Acc. Chem. Res.* **1980**, *13*, 317−323.

(41) Newcomb, M. *Tetrahedron* **1993**, *49*, 1151−1176.

(42) The temperature-dependent function for the reaction of secondary alkyl radicals with $(TMS)_3SiH$ is as follows:[43] $\log(k_H/M^{-1}\ s^{-1}) = 8.3 - 4.3/\theta$, where $\theta = 2.3RT$ kcal mol^{-1}.

(43) (a) Chatgilialoglu, C.; Dickhaut, J.; Giese, B. *J. Org. Chem.* **1991**, *56*, 6399−6403. (b) Chatgilialoglu, C.; Newcomb, M. *Adv. Organomet. Chem.* **1999**, *44*, 67−112.

(44) (a) Chatgilialoglu, C.; Ballestri, M.; Vecchi, D.; Curran, D. P. *Tetrahedron Lett.* **1996**, *37*, 6383−6386. (b) Haque, M. B.; Roberts, B. P.; Tocher, D. A. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2881−2889.

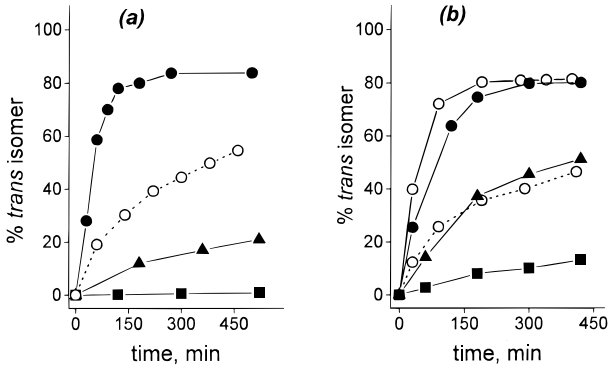


Figure 3. Time profiles of *trans*-isomer formation (i.e., methyl elaidate) in vesicles. (a) 0.15 M oleate contents and 30 mM AAPH at 37 °C: (○) MLVs with 75 mM $HOCH_2CH_2SH$; (●) LUVETs with 75 mM $HOCH_2CH_2SH$; (▲) LUVETs with 75 mM GSH; (■) LUVETs with 75 mM CySH. (b) 0.15 M oleate contents and γ-radiolysis (26.6 Gy min^{-1}) at 22 °C: (○, dotted line) MLVs with 75 mM $HOCH_2CH_2SH$; (○, solid line) LUVETs with 75 mM $HOCH_2CH_2SH$; (●) LUVETs with 7 mM $HOCH_2CH_2SH$; (▲) LUVETs with 7 mM GSH; (■) LUVETs with 7 mM CySH.

s^{-1} at −70 °C. Assuming log $A = 12.5$ s^{-1},[46] we calculated the β-elimination to be ca. 6×10^6 s^{-1} at 22 °C. This value, although associated with large errors, is in excellent agreement with the fact that the effectiveness and efficiency of *cis*−*trans* isomerization is uneffected by the presence of 0.2 mM oxygen. It is also worth underlining that the β-elimination of *n*-BuS• from radical **5** is about 1 order of magnitude higher than from radical **3**, suggesting that the β-methyl substantially decreases the activation energy due to the formation of a more stable olefin.[37]

Isomerization of DOPC in MLVs and LUVETs. As far as the model membranes are concerned, multilamellar vesicles (MLVs) and large unilamellar vesicles (LUVETs) made by the extrusion technique[47] were tested by both azo compounds and γ-irradiation using $HOCH_2CH_2SH$, GSH, or CySH as thiols. A DOPC/chloroform solution (3 mL; 0.15 mmol of oleate contents) was evaporated to a thin film in a test tube under an argon stream and then kept under vacuum for 30 min. A degassed phosphate-buffered saline was added, and MLVs were formed by vortex stirring for 7 min under an argon atmosphere. LUVETs were prepared by membrane extrusion with LiposoFast.

To MLV and LUVET suspensions were consecutively added the required amounts of $HOCH_2CH_2SH$ (0.075 mmol) and the water-soluble initiator AAPH (0.030 mmol). The samples were then warmed to 37 °C under argon, and aliquots (100 μL) were processed at different times. A comparison of the time profiles given by the two different vesicles is shown in Figure 3a (open circles for MLVs and solid circles for LUVETs). The efficiency

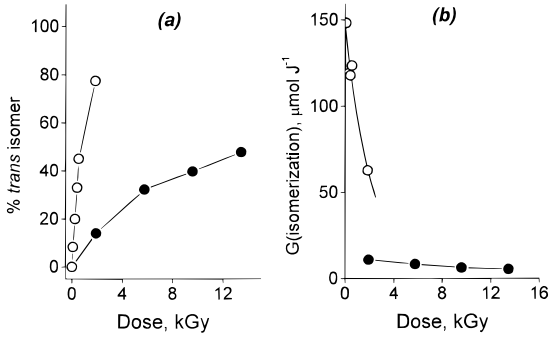


Figure 4. (a) *trans*-Isomer formation (i.e., methyl elaidate) vs dose from the γ-radiolysis of MLVs (0.15 M oleate contents) with 75 mM $HOCH_2CH_2SH$: (○) 1.4 Gy min^{-1}; (●) 30.6 Gy min^{-1}. (b) *G*(isomerization) vs dose: (○) 1.4 Gy min^{-1}; (●) 30.6 Gy min^{-1}.

of *cis*−*trans* isomerization decreased substantially by replacing LUVETs with MLVs. Isomerization can likely occur through the thiyl radicals, provided that they are able to migrate into the lipid compartment and attack the double bond of the fatty acid residues as described in Scheme 2. We suggest that the peculiar diffusional effects associated with each of the two differently organized systems are the main reason for the observed behavior. Figure 3a also shows the time profiles of GSH and CySH in LUVETs under identical conditions. Comparison of the three different thiols suggests that the isomerization rate follows the lipophilicity order of the three compounds[25] (i.e., $HOCH_2CH_2SH$ > GSH > CySH) and indicates that the CyS• radical is unable to migrate into the lipid compartment.

For γ-irradiation experiments, MLVs and LUVETs were prepared as described above and 2-propanol replaced the initiator in the aqueous compartment.[48a] The suspension was divided into different test tubes and flushed for 15 min with N_2O. Irradiation was then initiated, and at different times analysis of the progress of the reaction was carried out as already described. Figure 3b (open circles, dotted line for MLVs and solid line for LUVETs) shows the time profiles of $HOCH_2CH_2SH$ (75 mM). The efficiencies of *cis*−*trans* isomerization are similar to those observed by azo initiation experiments.

To model the biological environment, the amount of $HOCH_2CH_2SH$ concentration was decreased from 75 mM (open circles) to 7 mM (closed circles).[48b] It is gratifying to see that the effectiveness of the isomerization is the same, whereas the efficiency decreased only slightly. Figure 3b also shows the time profiles of GSH (7 mM) and CySH (7 mM) in LUVETs.[48] Again, the isomerization rate followed the lipophilicity order of the three thiols. The small amount of *trans*-isomer formation with CySH is not straightforward and will be discussed in detail in the next section.

Figure 4a shows the comparison of the experiments with MLVs and $HOCH_2CH_2SH$ under two different dose rates, i.e., 1.4 and 30.6 Gy min^{-1}. The higher efficiency of the *cis*−*trans* isomerization at a low dose rate is clearly due to the decrease in competitive reactions involving thiyl radicals. In fact, Figure 4b shows the plots of *G*(isomerization) vs dose for the two

(45) (a) The [**6**]/[*n*-BuSH] ratios of 0.146, 0.150, 0.178, 0.271, 0.263, and 0.374 were obtained for the $(TMS)_3SiH$ concentrations of 0.4, 0.6, 0.7, 0.8, 0.9, and 1.0 M, respectively. (b) Alternatively, linear regression analysis of [**6**]/[*n*-BuSH] vs [$(TMS)_3SiH$] gave $k_H/k_f = 0.37 \pm 0.09$ M^{-1}. (c) A detailed study of the β-elimination of RS• radicals from a variety of substituted radicals **2** (eq 11) using this methodology is in progress.

(46) β-Scission in radical chemistry has shown generally negative entropy of activation due to the reorganization necessary to achieve the transition state. For example, log *A* in the β-scission of the cumyloxyl radical is 12.4 s^{-1} (Baignée, A.; Howard, J. A.; Scaiano, J. C.; Stewart, L. C. *J. Am. Chem. Soc.* **1983**, *105*, 6120−6123), whereas for the decarboxylation of alkoxylcarbonyl radicals it is in the range of 12−13 s^{-1} (Beckwith, A. L. J.; Bowry, V. W. *J. Am. Chem. Soc.* **1994**, *116*, 2710−2716. Simakov, P. A.; Martinez, F. N.; Horner, J. H.; Newcomb, M. *J. Org. Chem.* **1998**, *63*, 1226−1232). For a general discussion on the preexponential factor, see: Benson, S. W. *Thermochemical Kinetics*, 2nd ed.; Wiley: New York, 1976.

(47) *Liposomes a practical approach*; New, R. R. C., Ed.; IRL Press: Oxford, 1990.

(48) (a) 2-Propanol was used to avoid reactions of •OH and •H species with moieties other than SH in the thiols. The rate constants for the reactions of •OH and •H species with the employed thiols are known to be close to 10^{10} and 10^9 M^{-1} s^{-1}, respectively.[27] To avoid changes in the lipid aggregates, we could not use 2-propanol as much as was necessary to capture all the •OH and •H. (b) On the basis of the available rate constants of the •OH and •H species with 2-propanol and the three thiols,[27] the 2-propanol concentration was chosen (0.23, 0.47, and 0.65 M for 7 mM $HOCH_2CH_2SH$, GSH, and CySH, respectively) so that ca. 90% of the •OH radicals and ca. 70% of the •H atoms reacted with 2-propanol (eqs 4 and 5).

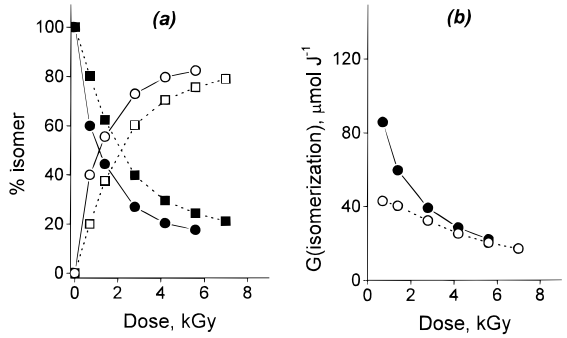


Figure 5. (a) Methyl oleate (●, ■) or methyl elaidate (○, □) vs dose from the γ-radiolysis (23.3 Gy min^{-1}) of methyl oleate in N_2O-saturated LUVETs at 22 °C: (●, ○) 0.15 M oleate contents; (■, □) 0.15 M oleate contents with 10% oxygen. (b) *G*(isomerization) vs dose: (●) without oxygen; (○) with oxygen.

different dose rate experiments. The extrapolation to zero dose gives *G* values of 150 and 12 μmol J^{-1} for the lower and higher doses, respectively. Taking[28] *G*(RS$^\bullet$) equal to 0.61 μmol J^{-1}, we calculated that each thiyl radical isomerized ca. 250 and 20 double bonds, respectively, at the initial phase.

Figure 5a (squares) shows the dose profile of the isomerization from LUVET/$HOCH_2CH_2SH$ saturated with 10% oxygen.[49a] For comparison, the same experiment without oxygen (circles) is also reported. The two reactions reached the same equilibrium mixture, but a longer time was required for the deoxygenated reaction. Figure 5b shows the plots of *G*(isomerization) vs dose which give *G*-values of ca. 120 and 45 μmol J^{-1} by extrapolation of the curve to zero dose for the reaction without and with oxygen, respectively. The decrease in *G* in the presence of 1.3×10^{-4} M oxygen[49a] can be explained as before; in fact, in the presence of 0.007 M thiol and 1.3×10^{-4} M oxygen, reaction 6 is only 5 times faster than the reaction of $Me_2C(^\bullet)OH$ with oxygen, and therefore a smaller concentration of thiyl radicals is produced.[27,49b] Comparison of Figures 2a and 5a evidences a decrease in efficiency in the *cis*−*trans* isomerization by the $HOCH_2CH_2S^\bullet$ radical in going from the homogeneous solution to LUVET experiments; the reason probably being that in LUVETs the radicals which are generated initially in the aqueous compartment have to migrate into the lipid compartment prior to isomerization. However, in LUVETs as happens in solution, the effectiveness of the *cis*−*trans* isomerization without or with 10% oxygen is the same.

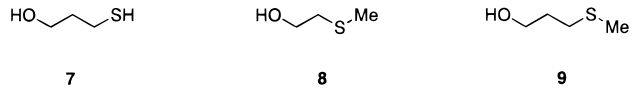
To address the question of whether the *cis*−*trans* isomerization could exhibit any positional preference in organized systems such as LUVETs, the fatty acid residues of the *sn*-1 and *sn*-2 positions of DOPC (Scheme 2) were analyzed at different isomeric compositions. Enzymatic hydrolysis by phospholipase A_2 from *Naja Naja* snake venom[50] specifically releases the fatty acid residue from the *sn*-2 position of the glycerol moiety and allowed us to separately examine the two positionally different hydrocarbon chains of the DOPC after isomerization. The lysophospholipid fraction was transesterified by treatment with methanolic potassium hydroxide, whereas the free fatty acid was transformed into the methyl ester by reaction with diazomethane. Table 1 shows the methyl oleate/methyl elaidate ratio

Table 1. Methyl Oleate/Methyl Elaidate Ratios of DOPC in LUVETs at Different Degrees of Isomerization

cis/*trans* ratio in DOPC	*cis*/*trans* ratio in the *sn*-1 position	*cis*/*trans* ratio in the *sn*-2 position
96/4	95/5	94/6
89/11	88/12	88/12
65/35	67/33	67/33
35/65	37/63	36/64

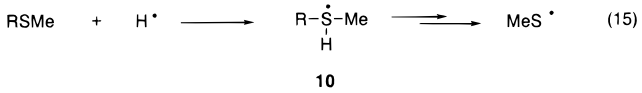
for each fraction as well as the total isomeric ratio in DOPC. In a number of experiments carried out, the isomerization was found to occur randomly without any positional preference.

Isomerization Using Other Sulfur-Containing Compounds. By replacing β-mercaptoethanol in *t*-BuOH solution with homo derivative **7**, similar time profiles of *cis*−*trans* isomerization were observed in both AMVN initiation and γ-irradiation (cf. Figure 2). However, when thiols were replaced by the corresponding *S*-methyl esters **8** and **9**, different behavior was observed.



7 **8** **9**

AMVN-initiatiated *t*-BuOH solutions containing sulfide **8** or **9** did not show any isomerization. The analogous experiments initiated by continuous radiolysis showed very slow isomerization; i.e., after 4 h the *cis/trans* ratio is smaller than 85/15 whereas the analogous experiments with thiols reached the equilibrium mixture of *cis/trans* = 17/83 in less than 2 h. We suggest that such behavior is due to the escape of H$^\bullet$ atoms from the radiolysis of *t*-BuOH and the subsequent reaction with sulfide **8** or **9** to form a sulfuranyl species, **10**, which in turn collapses to give indirectly MeS$^\bullet$ (eq 15), probably via the corresponding thiol.[51]



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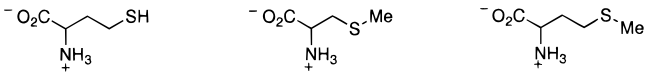
The *cis*−*trans* isomerization of DOPC in LUVETs was also tested with a variety of sulfur-containing compounds. In particular, the amphiphilic compounds **7**−**9** as well as the hydrophilic amino acid derivatives **11**−**13** were studied.[24] In



11 **12** **13**

Table 2 the *cis/trans* ratios after 3 h using AAPH as initiator or after 2 h using γ-radiolysis with 0.26 M 2-propanol are reported.[52] Azo-initiated isomerization is effective only in the case of amphiphilic thiol **7**. Neither the hydrophilic thiol **11** (cf. CySH in Figure 4a) nor the *S*-methyl derivatives **8**, **9**, **11**, and **13** are able to produce more than traces of the *trans*-isomer.

In the continuous radiolysis experiments, it can be seen that the amphiphilic thiol **7** behaves as expected (Table 2). On the other hand, the *S*-methyl derivatives of β-mercaptoethanol and cysteine (**8** and **12**) are able to promote substantial isomerization, whereas their homo derivatives **9**, **11**, and **13** are much less

(49) (a) This amount of oxygen corresponds to 1.3×10^{-4} M, which is ca. 3 times higher than that of typical well-oxygenated tissues, i.e., $[O_2] \cong 0.04$ mM. (b) Thiyl radicals have been reported to add reversibly to oxygen. For example, GS$^\bullet$ + $O_2 \rightleftarrows$ GSOO$^\bullet$, K = 3200 M^{-1}. Under our experimental conditions this reaction is considered to be unimportant. For a recent review, see: Wardman, P. In *S-Centered Radicals*; Alfassi, Z. B., Ed.; Wiley: Chichester, 1999; pp 289−309.

(50) Deems, R. A.; Dennis, E. A. *Methods Enzymol.* **1981**, *71*, 703−711.

(51) For reviews on sulfuranyl radicals, see: (a) Chatgilialoglu, C. In *The Chemistry of Sulphenic Acids and their Derivatives*; Patai, S., Ed.; Wiley: Chichester, 1990; pp 549−569. (b) Margaretha, P. In *S-Centered Radicals*; Alfassi, Z. B., Ed.; Wiley: Chichester, 1999; pp 277−288.

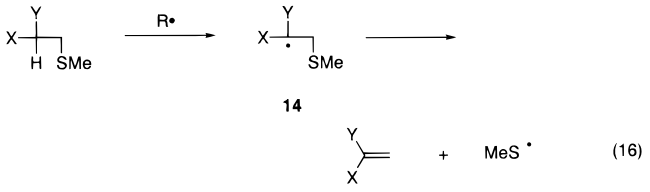
(52) Rationalizations similar to those in ref 48 are valid also for compounds **7**−**9** and **11**−**13**.

Table 2. Methyl Oleate/Methyl Elaidate Ratios of DOPC in LUVETs with a Variety of Sulfur-Containing Compounds

substrate	*cis*/*trans* ratio, AAPH/3 h[a]	*cis*/*trans* ratio, γ-irr/2 h[b]
7	23/77	18/82
8	99/1	33/67
9	99/1	75/25
11	98/2	88/12[c]
12	100/0	34/66
13	100/0	71/29

[a] 0.03 M initiator at 37 °C. [b] 0.26 M *i*-PrOH and 23.3 Gy min^{-1} at 22 °C. [c] 0.52 M *i*-PrOH.

effective. We suggest that under γ-radiolysis two different pathways contribute to the formation of thiyl radicals: (i) the escape of H$^\bullet$ atoms from the trapping of *i*-PrOH[48] and the subsequent reaction with sulfides (or thiols) to form a sulfuranyl species, **10**, which in turn collapses to give directly or indirectly the MeS$^\bullet$ radical (or HS$^\bullet$);[51,53] (ii) the initial radicals R$^\bullet$, which are more reactive than those derived from the thermal decomposition of AAPH, can abstract a hydrogen from positions that are activated by the neighboring groups. In the case of *S*-methyl derivative **8** or **12** the intermediate **14**, which is substantially stabilized by the α-substituents,[54] is in the β-position with respect to the SMe moiety. These radicals are expected to generate MeS$^\bullet$ radicals via β-elimination (eq 16).[55] Similarly the cysteine should form the HS$^\bullet$ radical. We also suggest that the MeS$^\bullet$ and HS$^\bullet$ radicals are able to migrate into the lipophilic compartment and isomerize the double bond.[56]



(16)

Fluidity of Model Membranes. To investigate the effect of the *trans* configuration on the physical properties of the lipid bilayer, experiments with TMA-DPH (a fluorescent probe of membrane fluidity) were performed. Figure 6 shows the temperature dependence of the steady-state fluorescence polarization of TMA−DPH[57a] assessed in MLVs of different compositions and determined within the range of 8−48 °C.

For dipalmitoyl phosphatidyl choline (DPPC) vesicles a sharp drop in fluorescence polarization is found at ca. 41 °C due to the liquid crystalline to fluid phase transition in agreement with literature data (open circles).[57b] On the other hand, experiments

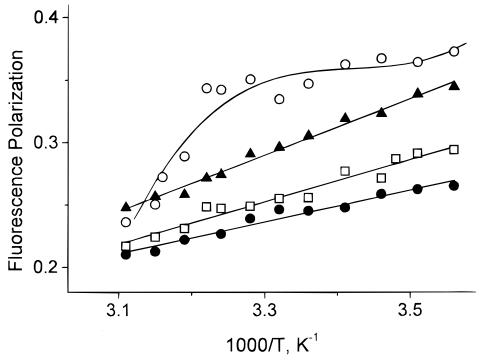


Figure 6. Polarization of TMA−DPH fluorescence as a function of temperature in (○) DPPC and (●) DOPC vesicles as well as in (□) DPPC/DOPC (50/50) and (▲) DPPC/DEPC/DOPC (50/40/10) vesicles.

with DOPC vesicles show that this model membrane exists in the fluid state over the temperature range studied (solid circles).

To test the dielaidoyl phosphatidyl choline (DEPC), we took the isomerized DOPC mixture, i.e., DOPC/DEPC (20/80), and prepared mixed vesicles of DPPC/DOPC (50/50) and DPPC/DEPC/DOPC (50/40/10). Figure 6 (open squares and solid triangles) shows that these model membranes also exist in the fluid state over the temperature range studied. However, the higher polarization values observed in DPPC/DEPC/DOPC vesicles show that the presence of *trans*-fatty acid residues substantially decreases the membrane fluidity.

Conclusions

We have shown that phospholipids containing *trans*-unsaturated fatty acids are the major products of the thiyl radical attack on natural phospholipids. We have also modeled the occurrence of such a reaction in oxygenated cell membranes using naturally occurring thiols. Although the isomerization process in lipid bilayer aggregates is somehow slower than in homogeneous solutions, it is still an effective process. Radicals can attack the double bond of the membrane lipids randomly, causing a change in the configuration of the hydrocarbon tails; the biological consequences can be predicted as they are very similar to the alterations already observed when *trans*-unsaturated fatty acids taken from the diet are included in membranes.[10]

Our results could also be seen as a new protocol for the conversion of "natural" phospholipids into their *trans*-isomers in solution, permitting different percentages of dielaidoyl phosphatidyl choline. Biotechnological application can be foreseen, for example, by preparing liposomes which contain a higher portion of *trans*-components and therefore show decreased permeability and increased stability against metabolic breakdown compared to the pure *cis*-analogues.[58]

The *cis*−*trans* isomerization of unsaturated lipids, together with autoxidation,[11] has to be considered when cellular damage caused by radical attack is examined since it can determine changes in barrier properties and functions of biological membranes. Therefore, accurate analyses of the cellular lipids are required. Furthermore, radiation can damage the membranes through the isomerization path, since thiyl radical species can be formed by naturally occurring sulfur-containing compounds in the biological enviroment.[59] The role played by thiols in this process is in antithesis to their action as radioprotectors.[62]

(53) The effect of ionizing radiations on sulfur-containing molecules of biological interest has been investigated for the last half-century. The following selection of references is of interest to this work: Jayson, G. G.; Stirling, D. A.; Shallow, A. J. *Int. J. Radiat. Biol.* **1971**, *19*, 143−156. Lal, M. *Can. J. Chem.* **1976**, *54*, 1092−1097. Lal, M. *Radiat. Phys. Chem.* **1982**, *19*, 427−434. Sjöberg, L.; Eriksen, T. E.; Revész, L. *Radiat. Res.* **1982**, *89*, 255−261. Hiller, K.-O.; Masloch, B.; Göbl, M.; Asmus, K.-D. *J. Am. Chem. Soc.* **1981**, *103*, 2734−2743. Zhao, R.; Lind, J.; Merényi, G.; Eriksen, T. E. *J. Am. Chem. Soc.* **1994**, *116*, 12010−12015. Goez, M.; Rozwadowski, J.; Marciniak, B. *J. Am. Chem. Soc.* **1996**, *118*, 2882−2891.

(54) Rauk, A.; Yu, D.; Armostrong, D. A. *J. Am. Chem. Soc.* **1998**, *120*, 8848−8855 and references therein.

(55) A N_2O-saturated water solution containing 0.1 M compound **12** was irradiated for 4 h. A 10% consumption of starting material was determined by ^{1}H NMR, and qualitative analysis showed the formation of CH_3SH, CH_3-CHO, CO_2, and NH_3.

(56) Collin, G. J.; Perrin, P. M. Garneau, F. X. *Can. J. Chem.* **1974**, *52*, 2337−2340.

(57) (a) TMA−DPH = 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene *p*-toluenesulfonate. (b) Andrich, M. P.; Vanderkooi, J. M. *Biochemistry* **1976**, *15*, 1257−1261.

(58) It is known that liposomes composed from phospholipids with saturated hydrocarbon chains yielded, for example, longer blood circulation, maintaining high biocompatibility. For a review, see: Lasic, D. D. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1685−1698.

(59) Radiation-induced lipid peroxidation was found to be correlated with membrane permeability,[60] but it was demonstrated that radiations did not affect monounsaturated lipids.[61]

Further work on the *cis*−*trans* isomerization of polyunsaturated phospholipids[21] by thiyl radicals and their biological implications is in progress.[63]

Experimental Section

Materials. DOPC and DPPC in chloroform (20 mg/mL), AIBN, AMVN, AAPH, PhSH, $HOCH_2CH_2SH$, CySH, GSH, 2-(methylthio)ethanol, 3-mercapto-1-propanol, 3-(methylthio)-propanol *S*-methyl cysteine, homocysteine, and methionine were commercially available from Aldrich, Fluka, or Sigma and used without further purification. TMA−DPH[57a] was purchased from Molecular Probes, Inc. (Eugene, OR). *tert*-Butyl alcohol and 2-propanol were purchased from Merck (HPLC grade) and used without further purification. *threo*-2-Bromo-3-butylthiobutane (**4**) was obtained by reaction of *cis*-2-butene with in situ prepared BuSBr,[64] whereas 2-butylthiobutane (**6**) was obtained by reaction of butyl thiolate with 2-bromobutane.[65]

General Methods. GC analyses for the determination of the isomeric ratio of the fatty acids were performed by using a Carlo Erba HRGC 5300 or Varian CP-3800 equipped with a flame ionization detector. As a stationary phase a Rtx-2330 column (60 m × 0.25 mm of 10% cyanopropylphenyl and 90% biscyanopropyl polysiloxane) was used with helium as the carrier gas. The heating was carried out at a temperature of 156 °C for 40 min followed by an increase of 10 °C/min up to 250 °C.[66] The methyl esters were identified by comparison with the retention times of authentic samples. GC analyses for the kinetic experiments were performed on an HP 5890 series II using a 30 m × 0.25 mm cross-linked 5% phenylsilicone capillary column (HP 5).

Continuous radiolyses were performed at room temperature (22 ± 2 °C) on 100 μL samples using a ^{60}Co-Gammacell at different dose rates. The exact absorbed radiation dose was determined with the Fricke chemical dosimeter, by taking $G(Fe^{3+}) = 1.61$ μmol J^{-1}.[67]

Measurements of steady-state polarization were performed with a Jasco FP-777 spectrofluorometer. The excitation and emission wavelengths were 362 and 429 nm, respectively. Values displayed by the instrument were averages of 10 measurements recorded during 20 s. Mean polarization (P) was calculated from these averaged measurements at each temperature according to the equation $P = (I_{VV} - I_{VH}F)/(I_{VV} + I_{VH}F)$, where I is the fluorescence intensity corrected for light scattering and the first and the second subscripts indicate the orientation, vertical (V) or horizontal (H), of the excitation polarizer and the emission polarizer, respectively. F is an optical correction factor (I_{HV}/I_{HH}).

Isomerization of Methyl Oleate in *tert*-Butyl Alcohol. A 0.15 M solution of methyl oleate in *t*-BuOH, containing either 0.075 M PhSH and 0.03 M AIBN or 0.075 M $HOCH_2CH_2SH$ and 0.03 M AMVN was heated at 71 or 54 °C, respectively. Alternatively, a N_2O-saturated *t*-BuOH solution of methyl oleate (0.15 M) was irradiated at 22 °C. Methyl esters were examined by GC analysis in comparison with the retention times of authentic samples. Methyl palmitate was used as the internal standard for quantitative studies.

Isomerization of DOPC in *tert*-Butyl Alcohol. A DOPC/chloroform solution (3 mL; 0.15 mmol of oleate contents) was evaporated in a test tube under an argon stream. *t*-BuOH (1 mL), $HOCH_2CH_2SH$ (0.075 mmol), and AMVN (0.030 mmol) were added, and the solution was degassed with argon. The reaction mixture was warmed at 54 °C, and aliquots (100 μL) were processed at different times. Alternatively, *t*-BuOH (1 mL) and $HOCH_2CH_2SH$ (0.007 mmol) were added, and the solution was divided into aliquots of 100 μL in different tubes followed by saturation with N_2O prior to γ-irradiation. Workup of the reaction aliquots was done by partitioning between *n*-hexane (or chloroform/methanol (2/1) in the case of vesicles) and brine, extraction and collection of the organic phases dried over anhydrous sodium sulfate, and evaporation of the solvent under vacuum at room temperature. The residue containing the phospholipids was treated with 0.5 M KOH/MeOH, for 10 min at rt, and then poured into the brine and extracted with *n*-hexane. The organic layer containing the corresponding fatty acid methyl esters was examined by GC analysis in comparison with the retention times of authentic samples.

Isomerization of DOPC in LUVETs. A DOPC/chloroform solution (3 mL; 0.15 mmol of oleate contents) was evaporated to a thin film in a test tube under an argon stream and under vacuum for 30 min. Degassed phosphate-buffered saline (PBS) (1 mL; Na_2HPO_4 (10 mM), NaCl (0.14 M), pH 7.2) was added, and MLVs were formed by vortex stirring for 7 min under an argon atmosphere. To obtain LUVETs, the lipid emulsion was transferred into LiposoFast (produced by AVESTIN, Inc.) and extruded 19 times back and forth through two polycarbonate membranes with a pore diameter of 100 nm.[47,68] To these suspensions were consecutively added the required amounts of thiol (0.075 mmol) and AAPH (0.030 mmol). The samples were then warmed to 37 °C under argon, and aliquots (100 μL) were processed at different times as previously described. For γ-irradiation experiments, LUVETs were prepared as described above in which 2-propanol (0.23−0.65 M depending on the nature of the thiol) replaced the initiator in the aqueous phase. The suspension was divided into different Pyrex test tubes, flushed for 15 min with N_2O, and irradiated at different times.

Regioselectivity of DOPC Isomerization. DOPC was isomerized at different *cis/trans* percentages using the above-reported procedure in LUVETs. The isomerization of fatty acids at the *sn*-1 and *sn*-2 positions of DOPC was analyzed by treatment with phospholipase A_2 from snake venom. Phospholipids (6.25 μmol) were evaporated under nitrogen and treated with 0.01 mg of phospholipase A_2 in 100 mM Tris−Cl, pH 8, containing 100 mM $CaCl_2$, 0.1 mg of bovine serum albumin, and 20 μmol of Triton X-100. The volume was brought to 1 mL with Tris buffer. Micelles were warmed at 37 °C for 1 h to ensure complete hydrolysis. At the end of the incubation, phospholipid dispersions were extracted three times with chloroform/methanol (2/1, v/v). The lower layers were collected and filtered through anhydrous sodium sulfate and the solvents evaporated under a nitrogen stream. Lipids were redissolved in chloroform, and the two hydrolysis products (fatty acids and 1-acyl-lysoPC) were separated by TLC on 0.2 mm thin silica gel coated plates with the solvent mixture chloroform/methanol/water (97.5/37.5/6.0,

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(66) It is worth noting that, under these conditions, it was also possible to separate the positional isomers of octadecenoic acid methyl esters and it was found that only Δ9 isomers are present in the reaction.

(67) Reference 29, p 100.

(68) The average diameter of the unilamellar vesicles was found to be ca. 90 nm; see: Fiorentini, D.; Cipollone, M.; Pugnaloni, A.; Biagini, G.; Landi, L. *Free Radical Res.* **1994**, *21*, 329−339.

v/v/v). The areas of silica gel corresponding to both the 1-acyl-lysoPC and the fatty acids were scraped off the plate and separately treated with 0.1 M KOH/MeOH for 10 min at rt and with diazomethane in ethyl ether, respectively, to convert the fatty acids into their corresponding esters for GC analysis.

Fluorescence Polarization Measurements. For fluorescence measurements multilayer vesicles of different compositions were prepared using PBS aliquots of the stock solutions. Vesicles of the following composition were obtained: 100 mol % DPPC, 100 mol % DOPC, 50 mol % DPPC and 50 mol % DOPC (50/50), and 50 mol % DPPC, 40 mol % DEPC, and 10 mol % DOPC (50/40/10). After complete removal of the solvents, first in a stream of nitrogen and then under vacuum, the lipids were suspended in PBS. The suspension was vortex-stirred thoroughly for 7 min at not less than 5 °C above the phase transition temperature of the lipid (the final concentration of lipid in the vesicles was 0.2−0.4 mg/mL). A 1 μL sample of a 1 mM solution of TMA−DPH in tetrahydrofuran was added to yield a final molar ratio of TMA−DPH relative to phospholipids of 1/650, and the samples were equilibrated for 40 min. Corresponding blanks were made according to the same procedure except that no TMA−DPH was added. Three labeled samples were prepared: two samples were used for measurements of fluorescence polarization as a function of temperature, and the third was used for correction of fluorescence depolarization due to light scattering.

For measurements of fluorescence polarization as a function of temperature, vesicles were first cooled from ambient temperature to 8 °C. The samples were then gradually heated by means of a circulating water bath. Fluorescence intensities were recorded after the samples were equilibrated at the desired temperature, which was held ±0.5 °C.

Kinetic Experiments for the β-Elimination of the *n*-BuS• Radical. Toluene containing a small amount of hexadecane as an internal GC standard was used as solvent. Bromide **4** (0.01 M) and $(TMS)_3SiH$ (0.4−1.0 M) were added, and the resulting solutions were degassed and photolyzed with a 500 W high-pressure mercury lamp at −70 °C for a few minutes. The products of interest, i.e., compound **6** and *n*-BuSH, were identified by comparison of their retention times with those of authentic materials.[45a]

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