The thiyl radical-mediated isomerization of *cis*-monounsaturated fatty acid residues in phospholipids: a novel path of membrane damage?

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Thiyl radicals, generated from biologically relevant thiols under biomimetic conditions, reversibly attack the double bonds of unsaturated phospholipids containing *cis*-fatty acid residues either in lipid solutions or lipid vesicles, thus producing phospholipids containing *trans*-fatty acid residues in high yield.

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, reported radical processes involving membrane phospholipids are limited to lipid peroxidation,[1] and to a recently reported homolytical cleavage of lysophospholipids.[2]

In cell membranes, the *cis* configuration of unsaturated fatty acid residues regulates the self-organization of phospholipids. The enzymatic *cis–trans* isomerization[3] has recently been found to affect the lipid assembly since the *trans* arrangement resembles the structure of saturated fatty acids.[4] From a chemical perspective, the *cis* to *trans* conversion of double bonds is a thermodynamically favoured process,[5,6] which can also occur by the reversible addition of a free radical.[7] In this context, thiyl radicals are very efficient isomerizing agents.[7,8] We report herein that thiyl radicals are also able to induce the isomerization of *cis*-monounsaturated phospholipids. This process was observed using lipid solutions and, more interestingly, liposome vesicles which model the cell membrane.[9] We have shown that phospholipids containing *trans*-fatty acid residues are produced in high yield.

Dioleoyl phosphatidyl choline (DOPC) was the substrate of choice since it represents the main natural component of cell membranes.[10] It is also known that the oxidizability of this substrate is very low.[11] A first set of experiments was carried out with a *tert*-butyl alcohol solution of DOPC, since it is known that phospholipids do not aggregate in this solvent.[12] A $CHCl_3$ solution of DOPC (3 ml; 0.15 mmol of oleate contents) was evaporated in a test tube under an argon stream. Bu^{t}OH (1 ml), $HOCH_2CH_2SH$ (0.075 mmol) and AMVN (0.030 mmol) were added and the solution degassed with argon.† The reaction mixture was warmed to 54 °C and aliquots (100 μl) were processed at different times. Alternatively, Bu^{t}OH (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.[13] After transesterification[14] of the phospholipids at different reaction times, the methyl oleate/methyl elaidate ratios were obtained by GC analysis. Fig. 1(*a*) and (*b*) show the time profiles of methyl elaidate (*i.e.* the *trans* isomer) formation. In all cases the conversion of the starting material to the equilibrium mixture was quantitative and in the absence of thiol the isomerization did not occur [Fig. 1(*a*) and (*b*)].

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 oleic acid residues, half-rotation about the carbon–carbon bond of the radical intermediate, and ejection of the thiyl radical by β-scission (Scheme 1).[7] It is worth pointing out that the final isomeric composition is in agreement with the difference in the thermodynamic stability between methyl elaidate and methyl oleate.[7]

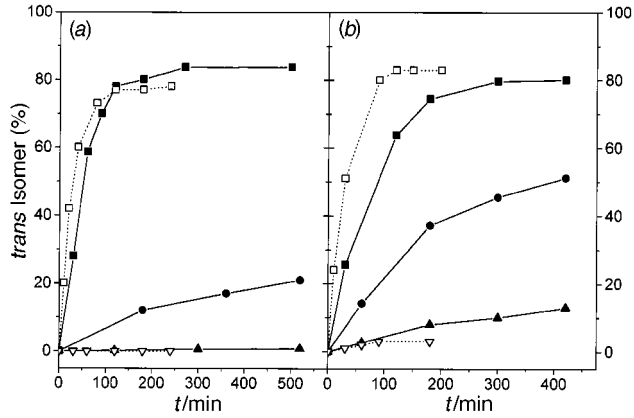
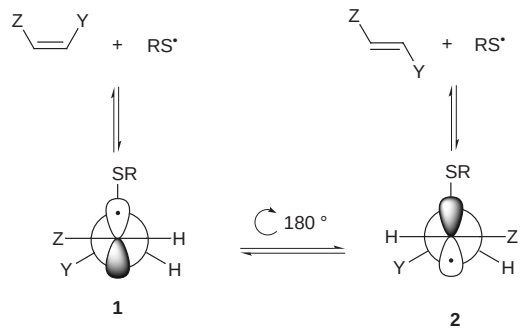


Fig. 1 Time profiles of *trans* isomer formation (*i.e.* methyl elaidate): (*a*) AMVN at 54 °C or AAPH at 37 °C: (□) DOPC with 75 mM $HOCH_2CH_2SH$ in Bu^{t}OH; (▽) DOPC without thiol in Bu^{t}OH; (■) LUVET with 75 mM $HOCH_2CH_2SH$; (●) LUVET with 75 mM of GSH; (▲) LUVET with 75 mM CySH. (*b*) γ-Radiolysis (32 Gy min^{-1}) at 22 °C: (□) DOPC with 7 mM $HOCH_2CH_2SH$ in Bu^{t}OH; (▽) DOPC without thiol in Bu^{t}OH; (■) LUVET with 7 mM $HOCH_2CH_2SH$; (●) LUVET with 7 mM GSH; (▲) LUVET with 7 mM CySH.

As far as the model membranes are concerned, large unilamellar vesicles (LUVET) made by the extrusion technique[15] were tested both by azo compounds and γ-irradiation using $HOCH_2CH_2SH$ or, alternatively, two other biologically-related thiols such as glutathione (GSH) and cysteine (CySH).‡

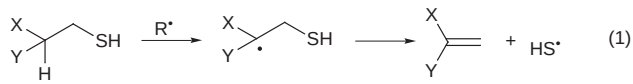
For the thermal initiation, we used the hydrophilic azo compound AAPH, by dissolving it with the thiol in the external



Scheme 1

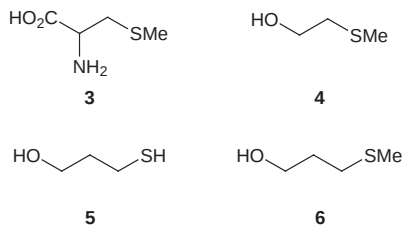
aqueous phase of the LUVET. A comparison of the time profiles given by the three different thiols is shown in Fig. 1(*a*). The isomerization is probably due to the thiyl radicals, provided that they are able to migrate into the lipophilic compartment, and attack the double bond of the phospholipids as described in Scheme 1. As a matter of fact, the isomerization rate follows the lipophilicity order of the three compounds[16] (*i.e.* $HOCH_2CH_2SH$ > GSH > CySH) and indicates that the CyS• radical is unable to induce isomerization.

For the γ-irradiation experiments the time profiles are shown in Fig. 1(*b*). The isomerization with CySH is not straightforward. In order to explain these results, we suggest that under γ-radiolysis the initial radicals,§ which are more reactive than those derived from the thermal decomposition of AAPH, can abstract either the thiol hydrogen or the β-hydrogen with respect to the SH moiety which is activated by the neighbouring groups [eqn. (1)].[17] We also suggest that the HS• radical obtained by β-



(1)

fragmentation is able to migrate into the lipophilic compartment and isomerize the double bond. The viability of eqn. (1) was confirmed by a series of control experiments. In particular, Fig. 2(*a*) shows the isomerization trends of LUVET with compounds **3** and **4** which are expected to generate MeS• radicals *via* a



reaction analogous to eqn. (1). On the other hand, using compounds **5** and **6**, isomerization occurs only with the former, the latter being inactive [Fig. 2(*a*)]. Fig. 2(*b*) shows a comparison of experiments with LUVET and $HOCH_2CH_2SH$ under two different dose rates. The higher efficiency of the *cis–trans* isomerization at a low dose rate is probably due to a decrease in competing reactions involving thiyl radicals.

In conclusion 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 modelled the occurrence of such a reaction in cell membranes using naturally occurring thiols. Furthermore, the role played by thiols in this process is in antithesis to their action as radioprotectors.[18] The *cis–trans* interconversion of unsaturated lipids has to be considered, together with autooxidation, when examining cellular damage caused by radical attack, since it can determine changes in barrier properties and functions of biological membranes. Therefore, accurate analyses of the cellular lipid content are required. Further work on the *cis–trans* isomerization of mono- and poly-unsaturated phospholipids and its biological implications is in progress.[19]

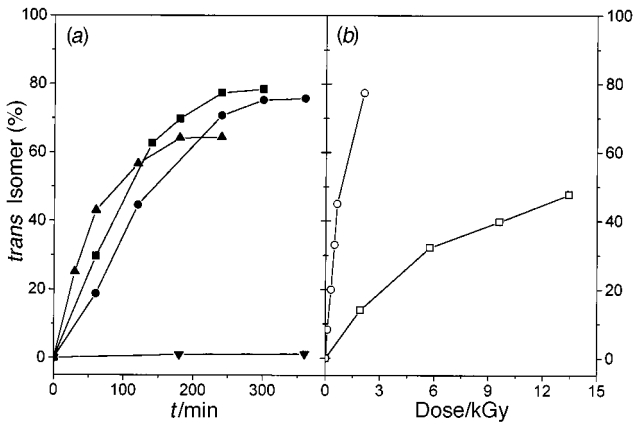


Fig. 2 (*a*) Time profiles of *trans* isomer formation (*i.e.* methyl elaidate) from the γ-radiolysis (32 Gy min^{-1}) of LUVET with (■ **3**, (●) **4**, (▲) **5** or (▼) **6** (all 7 mM). (*b*) *trans*-Isomer formation (*i.e.* methyl elaidate) *vs.* dose from the γ-radiolysis of LUVET with 7 mM $HOCH_2CH_2SH$.

Notes and references

† Dioleoyl phosphatidyl choline (DOPC) as a solution in $CHCl_3$ (20 mg ml^{-1}) and the radical initiators azobis(2,4-dimethylvaleronitrile) (AMVN) and azobis(2-amidinopropane) hydrochloride (AAPH) were commercially available and used without further purification.

‡ A $CHCl_3$ solution of DOPC (3 ml; 0.15 mmol of oleate contents) was evaporated to a thin film in a test tube under an argon stream. Degassed phosphate buffer (1 ml; Na_2HPO_4 10 mM, NaCl 0.14 M, pH 7.2) was added and MLV were formed by vortex stirring for 7 min under argon atmosphere. LUVET were prepared by membrane extrusion through 100 nm polycarbonate filters with LiposoFast™ (ref. 15). To these suspensions the required amounts of thiol (0.075 mmol) and AAPH (0.030 mmol) were consecutively added. The samples were then warmed to 37 °C under argon and aliquots (100 μl) were processed at different times. For γ-irradiation experiments, LUVET were prepared as described above in which Pr^iOH (0.12-0.35 mM depending from the nature of 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.

§ Radiolysis of water leads to species e_{aq}^- , HO• and H•. The presence of N_2O transforms the e_{aq}^- to HO• radicals. The presence of Pr^iOH transforms the majority of the above mentioned species into Me_2C•OH radicals which are believed to further react with thiols to generate thiyl radicals (ref. 13).

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