Biomimetic Chemistry

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The Sulfhydryl Radical ($HS^{\bullet}/S^{\bullet-}$): A Contender for the Isomerization of Double Bonds in Membrane Lipids**

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Some *trans* fatty acid residues found in living organisms can only be formed through an endogenous transformation of the naturally occurring *cis* structures, and their presence has been correlated with radical stress produced during physiological and pathological processes.[1–3] Diffusible thiyl radicals were deduced to be the species responsible for this isomerization on the basis of biomimetic models.[4–6] For example, methanethiyl radicals ($CH_3S^{\bullet}$) produced by the reductive modification of methionine residues promote the isomerization extremely efficiently.[7–9] Nitrogen dioxide ($NO_2^{\bullet}$) has also been suggested as an isomerizing species in a biological milieu;[2] however, it seems doubtful that $NO_2^{\bullet}$ really functions in this way.[4,6]

Hydrogen sulfide (H_2S) is emerging as an endogenously generated gaseous species with roles in the nervous and cardiovascular systems, and in pathological situations, such as inflammation and cerebral ischemia. Distinct enzymes are responsible for H_2S formation in the brain and the vascular system.[10–14] Physiological H_2S levels of 50–160 μM in mammalian brain tissue and 10–100 μM in human plasma have been reported. Chemically, the reactions of the simplest S-centered radical $HS^{\bullet}$ are not as well known as those of other thiyl radicals, although some key reactions in the aqueous phase have been characterized.[15–17] Different radicals and radical anions can be produced depending on the experimental conditions, but their roles as isomerizing agents are not known. From a biological perspective, H_2S-derived radical species in cellular pathways have not yet been reported, although the involvement of H_2S both in inflammation linked to radical stress and in cell signaling is suggestive of a complex reactivity. The role of H_2S as a precursor of diffusible S-centered radicals that act as isomerizing agents under biomimetic conditions is the subject of this study.

Our biomimetic model of a cell membrane consisted of liposomes made of a *cis* fatty acid containing a glycerophospholipid, namely, 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), in the form of large unilamellar vesicles (LUVET) of approximately 100 nm in diameter prepared by the extrusion technique.[18–20] A POPC-vesicle suspension (2 mM) in a phosphate buffer was saturated with N_2O or N_2, and a freshly prepared solution of Na_2S was added prior to γ radiolysis (dose rate of about 10.5 $Gy\,min^{-1}$) or photolysis (5.5-W low-pressure Hg lamp). After irradiation, lipid isolation, and derivatization to the corresponding fatty acid methyl esters,[21] the *cis*/*trans* ratio was determined by GC analysis.[18–20]

The solid lines in Figure 1 show the time profiles for the formation of *trans* fatty acid residues (elaidic (*trans*-9-octadecenoic) acid) upon photolysis of the vesicle suspension

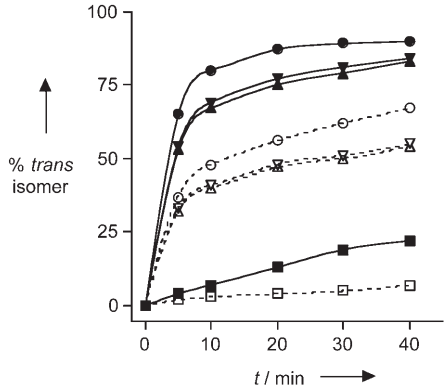

Figure 1. Formation of the *trans* isomer upon the photolysis of POPC vesicles (2 mM) in the presence of Na_2S (0.2 mM: solid lines; 1 mM: dashed lines) in a N_2-flushed phosphate buffer solution at various pH values: pH 3 (●, ○), pH 5 (▼, ▽), pH 7 (▲, △), pH 9 (■, □); the lines are interpolated from the data.

in the presence of Na_2S (0.2 mM) at different pH values. In all experiments the material balance after the geometrical isomerization of the POPC vesicles was quantitative. The efficiency of isomerization was influenced by the pH value; it decreased slightly when the pH value was increased from pH 3 (●) to pH 5 (▼) and pH 7 (▲), and was substantially lower at pH 9 (■). Surprisingly, at a higher concentration of H_2S, the efficiency of the isomerization dropped at all pH values. The dashed lines in Figure 1 describe analogous experiments in the presence of Na_2S at a concentration of 1 mM at pH 3 (○), pH 5 (▽), pH 7 (△), and pH 9 (□). The decrease in efficiency was found to be proportional to the increase in the concentration of H_2S at all pH values; for a fourfold increase in the quantity of Na_2S (from 0.2 to 1.0 mM), the formation of the *trans* isomer decreased by approximately 40%.

Similar results were obtained upon γ radiolysis of a suspension of POPC vesicles in the presence of H_2S. The inset in Figure 2 shows the irradiation–dose profiles of the disappearance of oleate residues (●) and the formation of

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elaidate residues (○) in the presence of Na_2S (0.1 mM) at pH 7. The dose profiles for the formation of the *trans* isomer at pH 7 and pH 5 are quite similar. The efficiency of *cis–trans* isomerization increased slightly at pH 3 and decreased substantially at pH 9. Again, when the concentration of Na_2S was increased, a proportional decrease in the efficiency of *cis–trans* isomerization was observed.

The UV photolysis ($\lambda = 250$–260 nm) of hydrogen sulfide (H_2S/HS^-, $pK_a = 6.89$) has been studied in some detail. Both forms afford the sulfhydryl radical [Eqs. (1) and (2)]. Hydrated electrons are trapped efficiently by H_2S [Eq. (3), $k_3 = 9.2 \times 10^9\,M^{-1}s^{-1}$][17] to yield H· atoms.

$$H_2S + h\nu \rightarrow HS^{\cdot} + H^{\cdot} \quad (1)$$

$$HS^- + h\nu \rightarrow HS^{\cdot} + e_{aq}^- \quad (2)$$

$$e_{aq}^- + H_2S \rightarrow H^{\cdot} + HS^- \quad (3)$$

The γ radiolysis of N_2O-saturated aqueous suspensions affords H· atoms (10%) and HO· radicals (90%) as the reactive species.[22] The total radiation chemical yield (G) of the two species is 0.61 μmol J^{-1}. Both species react readily with hydrogen sulfide with rate constants of $10^{10}\,M^{-1}s^{-1}$ [Eqs. (4) and (5)].

$$HO^{\cdot} + H_2S/HS^- \rightarrow H_2O + HS^{\cdot} \quad (4)$$

$$H^{\cdot} + H_2S/HS^- \rightarrow H_2 + HS^{\cdot} \quad (5)$$

The pK_a value of HS· is unknown, although evidence that S·$^-$ dominates in neutral aqueous solutions is strong [Eq. (6)]. Furthermore, S·$^-$ adds reversibly to HS^- to form the dimer radical [Eq. (7)], with forward and reverse rate constants of $k_7 = 4.0 \times 10^9\,M^{-1}s^{-1}$ and $k_{-7} = 5.1 \times 10^5\,s^{-1}$, respectively.[17]

$$HS^{\cdot} \rightleftharpoons S^{\cdot -} + H^+ \quad (6)$$

$$S^{\cdot -} + HS^- \rightleftharpoons HSS^{\cdot 2-} \quad (7)$$

The product yield (in mol kg^{-1}) divided by the absorbance dose (1 Gy = 1 J kg^{-1}) gives the radiation chemical yield or G(isomerization). Figure 2 shows the plot of G(isomerization) versus dose. Extrapolation to a dose of zero gives $G \cong 17$ μmol J^{-1}. We assumed a $G(HS^{\cdot}/S^{\cdot -})$ value of 0.61 μmol J^{-1} [Eqs. (4) and (5)] and calculated a catalytic cycle of isomerization to have a turnover number of 28 cycles in the initial phase. We suggest that HS·/S·$^-$ radicals in the aqueous compartment are able to migrate to the lipid bilayer and isomerize the double bond of the oleate moiety of POPC according to the catalytic mechanism shown in Scheme 1. The small degree of isomerization observed at a high pH value suggests that the dimer radical is the main species at pH 9; this radical is probably highly hydrophilic and/or unreactive towards the double bond of the oleate residue. On the other hand, the very similar behavior observed at pH 5 and pH 7 in both radiolytic and photolytic experiments suggests the involvement of the same isomerizing species, and indicates that the sulfhydryl radical has a pK_a value of approximately 4 (that is, a value three units lower than that of H_2S).

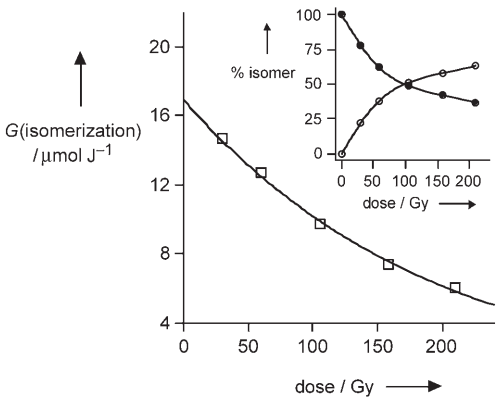
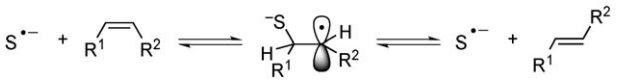


Figure 2. G(isomerization) versus dose; the line is the exponential fit to the data. Inset: Dose profile for the disappearance of oleate and formation of elaidate residues upon γ irradiation of POPC vesicles (2 mM) in the presence of Na_2S (0.1 mM) in an N_2O-saturated phosphate buffer solution at pH 7; the lines are interpolated from the data.



Scheme 1. Sulfhydryl radical catalyzed isomerization of *cis* phospholipids.

The isomerization induced by γ irradiation was then analyzed as a function of Na_2S concentration. Figure 3 (●) shows that for a dose of 105 Gy the formation of the *trans* lipid decreases linearly with an increasing concentration of Na_2S. This behavior is quite different to that observed for the analogous reactions with the $HOCH_2CH_2S^{\cdot}$ radical generated from the corresponding thiol at a dose of 100 Gy. Indeed, Figure 3 (○) shows that the percentage of the *trans* isomer is independent of the concentration of β-mercaptoethanol. even At concentrations higher than 60 μM and a dose of about 100 Gy, it is expected that both HO· radicals and H· atoms are scavenged by H_2S or $HOCH_2CH_2SH$.[21] Furthermore, a

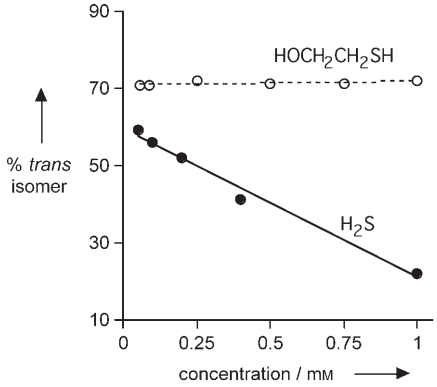


Figure 3. Formation of the *trans* isomer versus the concentration of Na_2S for the γ irradiation (105 Gy) of POPC vesicles (2 mM) in the presence of Na_2S in an N_2O-saturated phosphate buffer solution at pH 5. The data for analogous reactions (dose: 100 Gy) in the presence of $HOCH_2CH_2SH$ instead of Na_2S are also shown. The lines are a linear fit to the data.

catalytic cycle with a turnover number of about 60 is obtained for the $HOCH_2CH_2S^{\bullet}$ radical, whereas under identical conditions as used for the sulfhydryl radical the turnover number of 28 was calculated (see above). These findings suggest that by-products that can inhibit the *cis–trans* isomerization are formed in the reaction with H_2S.

On the topic of the buildup of inhibitors, it is well known that inorganic polysulfides (S_n^{2-} and their protonated forms) are important intermediates in the oxidation of H_2S, whereby the first step is thought to be the formation of the $HS^{\bullet}/S^{\bullet-}$ radical.[23,24] Indeed, after an irradiation dose of 420 Gy, the UV/Vis spectrum of reaction mixtures with an H_2S concentration of 0.4 mM contained new absorptions in the range 300–400 nm that are typical of S_n^{2-} ($n = 3$–8) and cyclic S_8 molecules.[25,26] S_8 was used to test the role of polysulfides as inhibitors of lipid isomerization. The dose profile for the formation of the *trans* isomer in suspensions of POPC vesicles at pH 7 in the presence of Na_2S (0.4 mM) was found to be identical to that observed with Na_2S at a concentration of 50 μM in the presence of S_8 (5 μM; data not shown). The mechanistic explanation for the inhibitor effect is based on electron transfer from the $S^{\bullet-}$ radical to polysulfides, a process which is likely to be exothermic [Eq. (8)]. The resulting

$$S_{n-1}^{2-} + S^{\cdot-} + H^+ \rightarrow S_{n-1}^{\cdot-} + HS^- \quad (8)$$

polysulfide radical anion can combine again with $S^{\bullet-}$ to form a higher polysulfide [Eq. (9)].[23] Thus, the polysulfide by-

$$S_{n-1}^{\cdot-} + S^{\cdot-} \rightarrow S_n^{2-} \quad (9)$$

products are good scavengers of the isomerizing agent, and when they reach a critical concentration, the isomerization is stopped completely.

In summary, we used a biomimetic model of vesicle suspension which mimics the aqueous and membrane compartments of a cell, and demonstrated the potential of sulfhydryl radicals ($HS^{\bullet}/S^{\bullet-}$) derived from H_2S to access the hydrophobic fatty acid chains and attack the double bonds. The phospholipids produced in this way contained a high proportion of *trans* fatty acid residues. This model offers some insight into the chemical basis of the biological activity of H_2S, which has not yet been established. This chemistry is important in view of the intriguing role of the sulfhydryl radical induced *cis–trans* conversion of lipids, either as a damaging or a signaling pathway.

Experimental Section

POPC was a gift from Chemi SpA (Cinisello Balsamo, Milan, Italy). LUVET were prepared as described previously[18,19] by using POPC with a final lipid or oleic acid content of 2 mM. The suspension (1 mL) was then transferred to a 4-mL vial equipped with an open-top screw cap and a teflon-faced septum, and saturated with N_2O or flushed with N_2. A stock solution of Na_2S in degassed phosphate buffer was freshly prepared for each experiment, and an aliquot was added to the suspension by syringe a few minutes prior to irradiation. Workup and analysis of the irradiated reaction mixture were carried out as previously reported.[18,19]

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