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Trans Lipids: The Free Radical Path

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ABSTRACT

Free radical-catalyzed cis−trans isomerization of unsaturated lipids and its outcome in biomimetic conditions are reviewed. They provided indications for an endogenous origin of trans unsaturated fatty acids in humans by the attack of thiyl radicals. A multidisciplinary approach highlights the relevance of this topic. The role of cis lipid geometry and its maintenance in the biological environment is an emerging question, with future developments in the fields of lipidomics, biology, and medicine.

Introduction

The role of lipids in cell metabolism is now fully recognized due to research progress in signaling activities and regulation of cellular processes. These functions are combined with the "old" but quite relevant and sometimes revisited roles of lipids as structural components of cell membranes and sources of metabolic energy.[1] Investigations into lipid repertoires and functions existing in living organisms have been refocused, and the field of "lipidomics" now assembles all of them.[2] Chemistry can contribute substantially with analytical protocols and synthetic methodologies to the ambitious project of mapping the entire lipidome and understanding the roles of lipids in health and disease.[3] Chemical studies into reactivity patterns and mechanisms involving lipids have already shown some relevant alterations occurring to these molecules in the biological environment. In particular, radical chemistry was fundamental for unveiling lipid reactivity. The radical-based peroxidation of polyunsaturated fatty acid residues in membrane and lipoprotein lipids has had an extraordinary biological meaning, since oxidative stress conditions now have a clear relationship with pathological situations[4] and the aging process.[5,6] The contribution of radical chemistry is currently extending to physiological conditions by the involvement of lipid radical species also during enzymatic activities.[7]

Our studies in radical chemistry related to lipids deal with the cis−trans isomerization of unsaturated moieties. When a double bond is considered in biology, it is ubiquitously found in the cis configuration, although cis isomer is thermodynamically less stable than its geometrical trans isomer. All structural and metabolic activities of mono- and polyunsaturated fatty acid moieties are based on the common feature of cis geometry (Scheme 1). The cis requisite is strictly controlled during lipid biosynthesis by desaturase enzymes, which in fact produce double bonds from saturated structures in a regiospecific and stereoselective manner.[8]

Research on cis−trans isomerization of double bonds in lipids embraces different disciplines. In the manufacturing of vegetable and fish oils, cis/trans isomeric mixtures of fats are obtained by partial hydrogenation and utilized in the food industry.[9] It is worth pointing out that in these cases the structures of trans fatty acid residues consist of geometrical and positional isomers having unshifted and shifted double bonds as compared to natural cis compounds. Nutritional and epidemiological studies have revealed some harmful effects of unnatural lipids for human health[9−11] and have motivated a crusade for amending food labeling in the U.S. by 2006, thus including trans fat information in the nutritional facts.[12]

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Carla Ferreri received a Laurea degree in Medicinal Chemistry from the University of Napoli in 1979. In 1984, she was appointed as researcher at the University of Napoli, and, since 2000, she is employed at Consiglio Nazionale delle Ricerche, Bologna. Her present research interests are in the field of organic synthesis and biomimetic models for studies of radical processes related to biological mechanisms.

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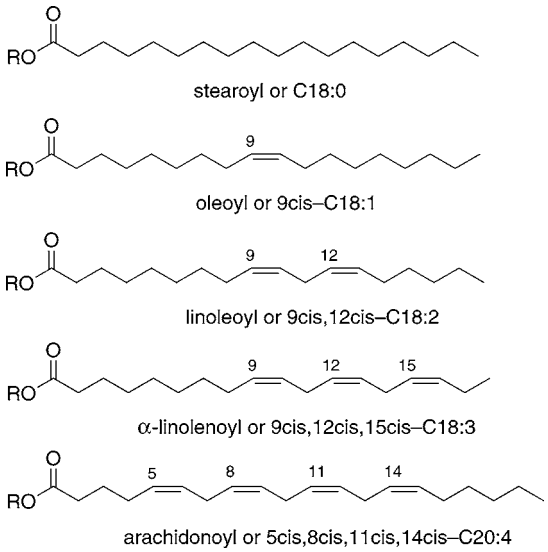
Scheme 1. Common Name and Numerical Abbreviation of Natural Fatty Acid Residues

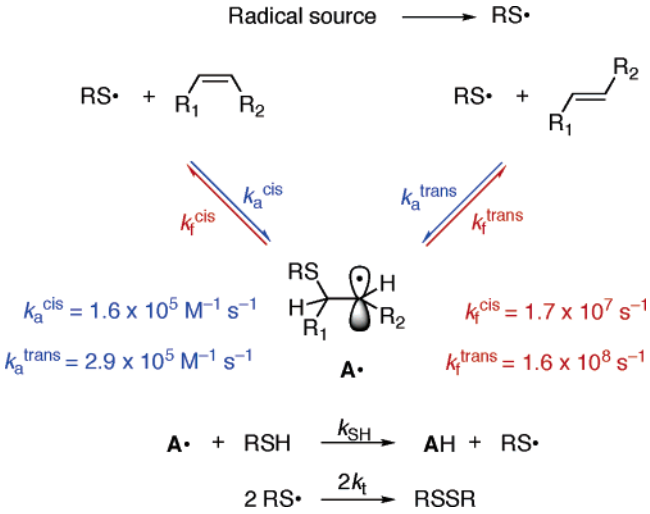


In microbiological research, it is known that geometrical trans lipids are formed in some bacteria by an enzymatic cis−trans isomerization during adaptation responses.[13,14] In biochemical investigations on the function and activity of trans isomers supplemented by diet, perturbation effects on cell membrane arrangement and lipid enzymatic cascades are indicated.[15−17] These findings resulted in a general view that trans isomers can occur in mammalian cells only after a dietary supplementation of chemically modified fats.

A few years ago, we thought that the chemical reactivity of double bonds under radical conditions could play a role in this scenario,[18] especially considering that thiyl radicals RS• are among the most effective species known to catalyze the cis−trans isomerization process,[19,20] and that thiyl radicals are generated from thiols under the biological conditions of radical stress.[21,22] We reconsidered the trans geometry in natural lipids, and as the first issue we addressed a crucial question: can trans lipids be generated from two different pathways, from food intake (exogenous path), and from a radical-catalyzed isomerization occurring in a biological context (endogenous path)? The answer could have had an additional meaning in view of a comprehensive chemical biology approach, which correlates the lipid structural change with perturbing biological effects.

Our research strategy combined several aspects: (i) mechanistic and product studies on lipid isomerization in homogeneous and heterogeneous systems, for gathering quantitative data on the isomerization steps and product formation; (ii) design of biomimetic models such as model membranes, to study trans lipid formation in the presence of biologically meaningful sulfur-containing compounds and different radical initiations; (iii) synthetic studies for building up a trans lipid library to be used in lipidomics and pharmacological research as well as addressing the exogenous/endogenous path discrimination; (iv) vesicle studies for showing the effect of the trans geometry in lipid assembly; and (v) studies of biological

Scheme 2. Reaction Mechanism for the Cis−Trans Isomerization Catalyzed by Thiyl Radicals (Rate Constants at 25 °C)



systems under radical stress conditions for testing the endogenous path. An overview of our achievements and trends of future developments will be presented in this Account.

Geometric Isomerization of Unsaturated Fatty Acids Catalyzed by Thiyl Radicals

Monounsaturated Fatty Acids (MUFA). The early work of the addition of thiols to olefins was accurately summarized in 1970 by Griesbaum.[23] The reaction was discovered 100 years ago by Posner, although its formulation as a free radical chain process was achieved by Kharasch and co-workers just before the Second World War. This reaction has attracted considerable attention in organic synthesis.[24] Thiol additions to cis and trans olefins are accompanied by isomerizations, and this has led to the general mechanism shown in Scheme 2.[19] That is, thiyl radicals (RS•) add reversibly to the double bond to form radical adduct **A**•, which undergoes chain transfer with thiol to form the adduct **A**H. In terms of cis−trans isomerization, the RS• radical acts as a catalyst. Recently, the time-dependence of the $HOCH_2CH_2SH$ addition to methyl oleate and of the isomerization catalyzed by $HOCH_2CH_2S$• radical was investigated in detail by varying the method of thiyl radical generation.[25,26] Figure 1 shows an example of time profiles of the disappearance of methyl oleate (red ●), and the formation of methyl elaidate or 9trans−C18:1 (red ○). Their sum (red ◑) indicates loss of unsaturation that is exactly balanced by the formation of the thiol adduct (blue ▼). Indeed, material balance (green ■) is constant during the reaction course. The kinetics was analyzed on the basis of the initiation rates and the known values of the thiyl self-termination rate constant ($2k_t$) and rate of hydrogen abstraction from the thiol (k_{SH}), providing room-temperature rate constants for all four reactions involved in the isomerization (see Scheme 2). The rate constants for RS• addition (k_a in blue) to cis or trans isomers were found to be rather similar ($k_a^{trans}/k_a^{cis} = 1.8$), whereas for fragmentation of **A**• (k_f in red) they were substantially different ($k_f^{trans}/k_f^{cis} = 9.4$). The large prefer-

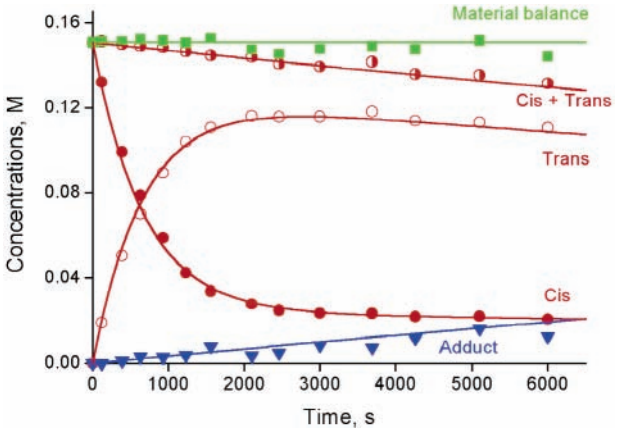


FIGURE 1. Cis–trans isomerization of methyl oleate (150 mM) by thiyl radicals generated by photolysis of di-*tert*-butyl ketone (37.5 mM) and β-mercaptoethanol (222 mM) in *tert*-butyl alcohol at 20 ± 2 °C and formation of the thiol adduct.

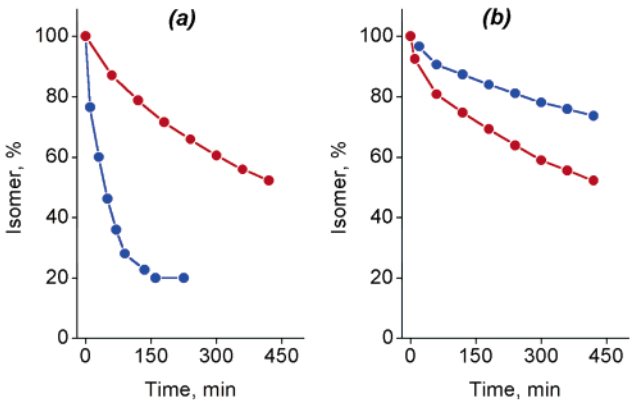
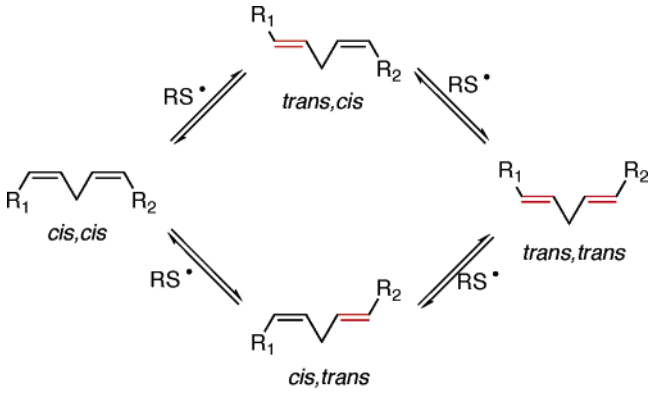


FIGURE 2. Time profiles of methyl linoleate (red ●) or methyl oleate (blue ●) in *tert*-butyl alcohol at 54 °C. (a) Isomerization of methyl linoleate (0.15 M) with 75 mM $HOCH_2CH_2SH$ and 30 mM AMVN, or methyl oleate (0.15 M) with 75 mM $HOCH_2CH_2SH$ and 30 mM AMVN. (b) Isomerization of a mixture of methyl linoleate (0.15 M) and methyl oleate (0.15 M) with 75 mM $HOCH_2CH_2SH$ and 30 mM AMVN.

Scheme 3. Cis–Trans Isomerization of PUFA Catalyzed by Thiyl Radicals

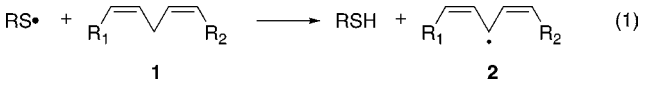


ence of fragmentation to the trans isomer was attributed to different barriers for the formation of the two transition states from the equilibrium radical structure. Similar results were obtained for a variety of MUFA, indicating that these reactions do not depend on the double bond position in the alkyl chain.[27] The equilibrium constant of $K = 5.15$ for MUFA is larger than that of the 2-butenes ($K = 3.4$), presumably because of the larger alkyl chain residues. A temperature dependence of K for oleate in *tert*-butyl alcohol provides $\Delta H = -5.4$ kJ/mol and $\Delta S = -5.5$ J/(mol K) for the cis to trans conversion.[25]

Polyunsaturated Fatty Acids (PUFA). Although the understanding of MUFA isomerization is quite clear, the same cannot be said for the analogous reactions of PUFA. Each isolated double bond in PUFA should behave independently as discussed in the previous section. Indeed, the time profiles of methyl linoleate disappearance and formation of mono-trans and di-trans isomers in these experiments indicated that the cis–trans isomerization occurs stepwise (Scheme 3).[28] Cis–trans isomerization reactions of methyl γ-linolenate and arachidonate catalyzed by $HOCH_2CH_2S^\bullet$ radicals have also been studied in some detail.[28,29] The number of possible geometrical isomers increases according to 2^n, where n is the number of double bonds, and the complete analysis may be difficult and incomplete for high unsaturation as in the case of methyl arachidonate.[29] However, the step-by-step mechanism reported in Scheme 3 also governs the isomerization of these highly unsaturated fatty acids.

Side reactions are also at work. Figure 2a shows the time profile of the disappearance of methyl linoleate (red ●) and methyl oleate (blue ●) under identical conditions (thermal initiation using AMVN at 54 °C) for comparison, where the isomerization of methyl linoleate was unexpectedly much slower. On the other hand, Figure 2b shows the isomerization of a mixture of the two esters, where methyl linoleate (red ●) isomerized 2 times faster than methyl oleate (blue ●), as expected. The efficiency of methyl linoleate isomerization is the same in the presence or absence of methyl oleate, whereas the disappearance of methyl oleate is dramatically decreased in the presence of methyl linoleate. Product studies of methyl linoleate isomerization showed that the four geometrical isomers (Scheme 3) account for 93% of the material balance together with the formation of byproducts containing "conjugated diene" moieties, which act as inhibitor for the cis–trans isomerization.[28]

PUFA (**1**) have methylene-interrupted double bonds, and the bisallylic positions have quite a low C–H bond dissociation enthalpy, so that the reaction with alkanethiyl radicals ($RS^\bullet$) is exothermic (eq 1). Pentadienyl radicals **2** are very well documented, being intermediates in lipid peroxidation.[30] An intense absorption around 280 nm is characteristic of their transient UV spectra. The reactions of a variety of biologically relevant $RS^\bullet$ radicals with PUFA have been investigated by pulse radiolysis following the buildup of the transients.[31] For example, rate constants in the range $(0.6–3.1) \times 10^7$ M^{-1} s^{-1} were obtained for linoleic acid. Based on these findings, early product studies of cis–trans isomerization of methyl linoleate were interpreted as an outcome of the fate of pentadienyl radical, which reacts with thiols in different conformations.[32] This scenario is unlikely because the conformational barriers of pentadienyl radicals are rather large.[30]



(1)

1 2

The time-resolved experiments are still odd in the sense that the overlap with product studies is not straightforward. If eq 1 is 2 orders of magnitude faster than the thiyl radical addition, it should be reversible or the observed transient is something else that resembles the pentadienyl radical. Experiments on the reaction of methyl linoleate with $HOCH_2CH_2S^•$ radical are currently underway in our laboratory to support or deny the intermediacy of pentadienyl radical in these reactions.

The Presence of Molecular Oxygen. Cis−trans isomerization reaction of MUFA or PUFA was also successfully reported in the presence of 0.1−0.3 mM of molecular oxygen, which is a few times higher than the molarity of typical well-oxygenated tissues, that is, $[O_2] = 0.04$ mM. For example, the $HOCH_2CH_2S^•$ radical-induced cis−trans isomerization of methyl oleate[33] or methyl linoleate[28] in *tert*-butyl alcohol solutions saturated with 10% oxygen (corresponding to 0.23 mM) behaved as it did without oxygen.

How are these results placed with regards to the well-known co-oxidation reaction of thiols with olefins?[23,34] In the three-component reaction (thiol/olefin/oxygen), the intermediate alkyl radical **A**• in Scheme 2 should combine with O_2 and the resulting peroxyl radical either undergoes chain transfer with thiol to form a hydroperoxide[23] or, in the case of PUFA,[35] cyclizes to the nearest double bond to give 1,2-dioxolane as the final product. The extent to which the reversibility of thiyl radical addition plays a role is dependent on a variety of factors. The most noteworthy example is the β-elimination of thiyl radical from alkyl radical, which does not carry a second β-substituent. Indeed, the rate constant for the β-fragmentation of $RSCH_2CH(•)CH_2R$ radical is 2.5×10^6 s^{-1} at 25 °C,[25] that is, about 1 and 2 orders of magnitude slower for the formation of cis and trans isomers, respectively, in Scheme 2. For the analogous aromatic substituted $ArSCH_2CH(•)CH_2R$ radical, a value of $\sim 1 \times 10^7$ s^{-1} can be estimated.[36] An inspection of the co-oxidation data in the literature[23,34] indicated the use of terminal and/or conjugated olefins with preferably aromatic thiols under high oxygen concentrations. Therefore, at oxygen concentrations lower than 0.3 mM, the co-oxidation path in the cis−trans isomerization of unsaturated fatty acid residues is unimportant because of very fast β-elimination of thiyl radicals, that is, $k_f^{trans} \gg k_f^{cis} \gg k_{oxygen}[O_2]$.

The Influence of Antioxidant Vitamins. The effectiveness of cis−trans isomerization in the presence of the most common antioxidants has been addressed.[27] The high reactivity of RS• radical addition to all-trans retinol (1.4×10^9 M^{-1} s^{-1} for GS•) and β-carotene (2.5×10^9 M^{-1} s^{-1} for $HOCH_2CH_2S^•$) suggested that retinoid and carotenoid derivatives are the best inhibitors of the cis−trans isomerization.[37] Figure 3 shows the dose profile of disappearance of methyl oleate (blue ●) during the cis−trans isomerization catalyzed by radiolytically generated $HOCH_2CH_2S^•$ radicals. The same experiment in the presence of 1 mM of all-trans retinol acetate **3** (red ●) shows an initial strong inhibition of the isomerization, although the cis/trans isomeric ratio reached the same equilibration point in a longer time, which means that the thiyl radicals are efficiently scavenged from the conjugated diene until it is consumed. Similar experiments by replacing the conjugated diene with α-tocopherol or ascorbic acid 6-*O*-palmitate showed that thiyl radicals are weakly scavenged by these two antioxidants.[27]

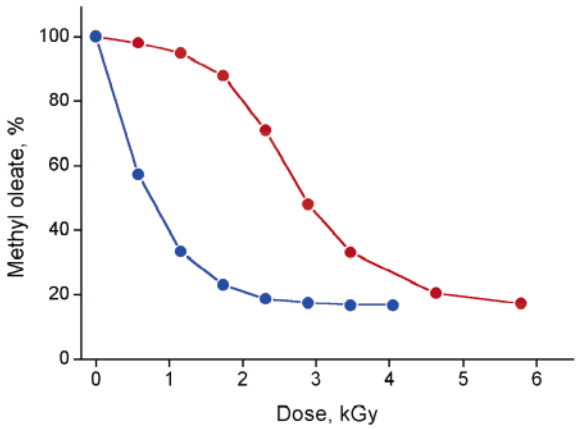
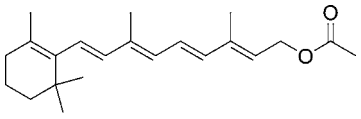


FIGURE 3. Effect of all-trans retinol acetate on the isomerization of methyl oleate. γ-Radiolysis of methyl oleate (0.15 M) in N_2O-saturated *tert*-butyl alcohol with 75 mM $HOCH_2CH_2SH$ at 22 °C in the absence (blue ●) and in the presence of 1 mM all-trans retinol acetate (red ●).



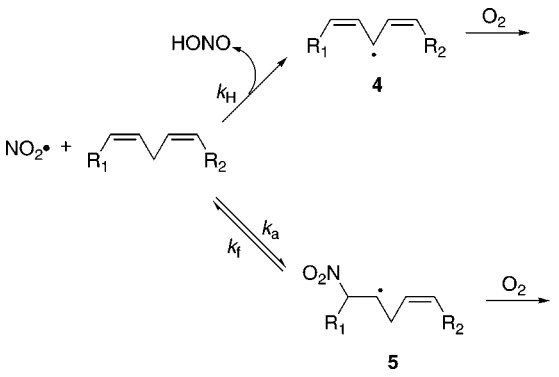
3

Geometric Isomerization of Unsaturated Fatty Acids Catalyzed by Nitrogen Dioxide

Other radicals and atoms such as Br•, I•, $RSO_2^•$, $NO_2^•$, etc., are known to induce cis−trans isomerization of double bonds by addition−elimination steps. The most relevant species from a biological point of view is nitrogen dioxide ($NO_2^•$) for which Titov first noted activity in geometric isomerization in 1963.[38] Reliable kinetic data for the reaction of $NO_2^•$ radical with olefins are limited to the gas phase, because both addition and hydrogen abstraction reactions are very slow processes.[39] For example, the rate constants at 25 °C for addition of $NO_2^•$ to 2-pentene are $k_a^{cis} = 0.18$ and $k_a^{trans} = 0.038$ M^{-1} s^{-1}, whereas the fragmentation of the adduct radical occurs with $k_f \approx 8 \times 10^4$ s^{-1} for both isomers. Evidence that H-atom abstraction from the allylic position (~20 kJ/mol endothermic) is of the same order of magnitude as the addition step is also available. A detailed study of $NO_2^•$ with cyclohexene by Pryor and co-workers showed that the two reaction paths are also competitive in solution.[40]

The reaction of $NO_2^•$ with methyl linoleate in the presence and in the absence of oxygen has attracted considerable attention because the $NO_2^•$ radical has been proposed to initiate lipid peroxidation.[41] The rate constant for the abstraction of bisallylic H-atom by $NO_2^•$ (almost

Scheme 4. Hydrogen Abstraction versus Addition Mechanism for the Reaction of Nitrogen Dioxide with PUFA



thermoneutral) is expected to be faster than the abstraction from the allylic positions. Scheme 4 shows the hydrogen abstraction versus addition mechanism for the reaction of $NO_2^\bullet$ with PUFA that fits the experimental findings.[40,42] The reaction products strongly depend on the reaction conditions. At low $NO_2^\bullet$ concentrations, for example, hydrogen abstraction predominates under anaerobic conditions with the formation of allylic products derived from radical **4** and small yields of trans isomers, whereas in the presence of oxygen trans isomers were not detected due to the trapping of radical **5** by oxygen.[42] Therefore, in the presence of 0.1 mM oxygen, the cis–trans isomerization becomes unimportant, because both paths generate carbon-centered radicals that react with O_2 to give peroxyl radicals that propagate peroxidation.

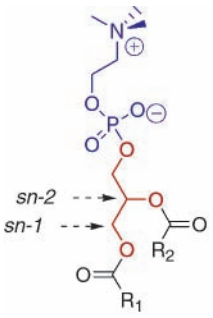
$NO_2^\bullet$ is also a moderate one-electron oxidant, $E^\circ(NO_2^\bullet/NO_2^-) = 1.04$ V (vs NHE). At pH 7.4, rate constants for reaction of $NO_2^\bullet$ with glutathione and cysteine were estimated to be close to 2×10^7 M^{-1} s^{-1}.[43] The variation of these rate constants with pH indicated that thiolate anion reacted much faster than undissociated thiol. As Wardman and co-workers underlined,[43] the implication of these values is that thiols are the dominant "sink" for $NO_2^\bullet$ in cell/tissue, whereas urate is the major scavenger of $NO_2^\bullet$ in plasma.

Isomerization of Triglycerides in Solution

The unsaturated fatty acid moieties of triglycerides contained in natural oils (borage, olive, and rice) were converted to their corresponding geometrical trans isomers by the action of photolytically or thermally generated $HOCH_2CH_2S^\bullet$ radical in alcoholic solutions.[44] It is worth pointing out that during partial hydrogenation processes of edible oils, a fraction of the unsaturated fatty acid moieties is converted to trans isomers that contain predominantly positional isomers.[45]

The RS• radical-catalyzed approach was designed for building-up a simple and economic *Trans Lipid Library* with application to lipidomics.[44] Oils obtained from the isomerization may be treated by winterization, to further increase the trans FA content. Hydrolysis or methanolysis of trans triglyceride mixtures were enzymatically carried out to obtain the corresponding trans FA or trans FA methyl esters. These results are relevant for the studies

Scheme 5. Structure and Composition of Fatty Acid Residues of L-α-Phosphatidylcholines (PC) Used in This Account



Soybean Lecithin	
Palmitoyl	4.5%
Stearoyl	3.8%
Oleoyl	11.9%
Vaccenoyl	1.3%
Linoleoyl	63.5%
γ-Linolenoyl	6.3%

Egg Lecithin	
Palmitoyl	32.0%
Stearoyl	14.1%
Oleoyl	27.0%
Vaccenoyl	1.2%
Linoleoyl	20.0%
Arachidonoyl	4.8%

DOPC: $R_1 = R_2$ = Oleoyl
SAPC: R_1 = Stearoyl, R_2 = Arachidonoyl

of lipid isomerism and trans fatty acid recognition and open new perspectives for the synthesis of glycerides and studies of their structure–activity relationships.

Isomerization of L-α-Phosphatidylcholines (PC)

In L-α-phosphatidylcholines (PC), the most abundant phospholipids in nature, a three-carbon glycerol bridge links the two fatty acid chains with a phosphoryl choline moiety. Scheme 5 shows the three distinct regions of PC in different colors. In the *sn-1* and *sn-2* positions of the glycerol moiety, the two fatty acid residues are attached, whereas in the *sn-3* position the polar headgroup is connected. DOPC (dioleoyl phosphatidylcholine) and SAPC (stearoyl arachidonoyl phosphatidylcholine), together with soybean and egg yolk lecithins, are used in this Account. Lecithin from natural sources is a mixture of L-α-phosphatidylcholines with different fatty acid chains. Detailed studies of cis–trans isomerization catalyzed by RS• radicals have been carried out on these phospholipids in homogeneous solution and in large unilamellar vesicles (liposomes).

Isomerization of PC in Solution. The cis–trans isomerization of unsaturated fatty acid residues of DOPC,[33] SAPC,[46] soybean lecithin,[28] and egg lecithin[46] catalyzed by $HOCH_2CH_2S^\bullet$ radicals was studied in alcoholic solutions. After transesterification of the phospholipids, the FA methyl esters composition was obtained by GC analysis. The results can be summarized as follows: (i) the efficiency of isomerization depends mainly on the method of generation of thiyl radicals; (ii) for a particular fatty acid, the isomerization trend is similar to the analogous isomerization of fatty acid methyl esters; that is, it produces only geometrical isomers; (iii) for PUFA residues, it is envisaged from the isomerization profile of mono-trans and di-trans isomers that the step-by-step mechanism shown in Scheme 3 operates; and (iv) all of the cis double bonds isomerized with the same efficiency independently of their location. The last observation is clearly illustrated in the experiment with egg lecithin, where the time courses of the disappearance of oleate (green ●), linoleate (red ●), and arachidonate (blue ●) residues are normalized to 100% for a better comparison (Figure 4a). In the initial stage of the reaction, the arachidonate residue (having four double bonds) isomerized 2 times faster than linoleate residue

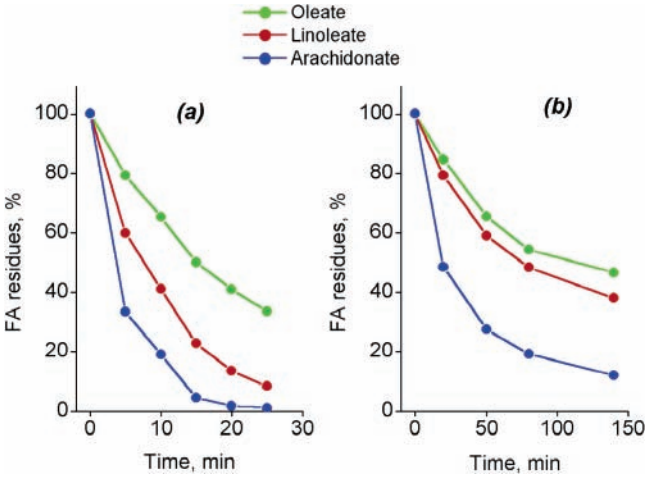


FIGURE 4. Time profiles of the disappearance of cis fatty acid residues obtained by photolysis of egg yolk lecithin in 2-propanol (a) and in LUVET (b). Conditions: 15 mM fatty acid contents with 7 mM $HOCH_2CH_2SH$ at 22 °C.

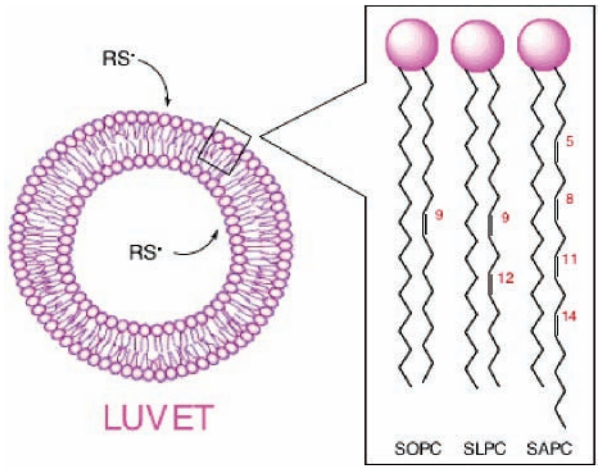


FIGURE 5. Schematic representation of large unilamellar vesicles made by the extrusion technique (LUVET) and three potential phosphatidylcholines (SOPC, SLPC, and SAPC). Phospholipid diacyl chains are drawn parallel for simplicity, and the carbons of the C−C double bond are numbered sequentially from the carboxyl end that is contained in the polar headgroup. Thiyl radicals (RS•) are initially generated in the aqueous compartment and may enter the lipid bilayer.

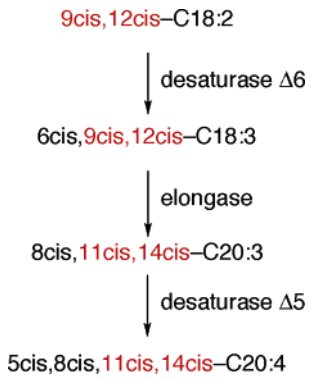
(having two double bonds) and 4 times faster than oleate residue (having one double bond). It is also worth underlining that the two mono-trans isomers of linoleate residues were formed in the same amounts, as it occurs with the four mono-trans isomers of arachidonate residues. Evidence that the effectiveness of the cis−trans isomerization in the presence of 0.1−0.2 mM of oxygen is the same has also been obtained in the experiments with SAPC[46] and soybean lecithin.[28]

Isomerization of PC in Large Unilamellar Vesicles. Initial studies of cis−trans isomerization of unsaturated moieties of DOPC vesicles catalyzed by thiyl radical were performed under a variety of conditions.[18,33] By varying the lipophilicity of thiyl radical and its method of generation, multilamellar vesicles (MLV) and large unilamellar vesicles made by the extrusion technique (LUVET) were tested. It was shown that the isomerization rate followed the lipophilicity order of thiols as well as the complexity of the supramolecular organization. Amphiphilic $HOCH_2CH_2SH$ in LUVET gave the highest efficiency of geometric isomerization, indicating that thiyl radicals generated in the aqueous compartment enter the hydrophobic region of the bilayer and isomerize the 9,10-double bonds of oleic moieties (cf. left part of Figure 5). The presence of 0.2 mM oxygen did not influence the effectiveness of this geometric isomerization. Evidence that no positional preference for isomerization of fatty acid residues of *sn-1* and *sn-2* positions of PC (cf. Scheme 5) was also obtained for DOPC in LUVET catalyzed by $HOCH_2CH_2S^{\bullet}$ radicals.[33]

The isomerization of soybean lecithin in LUVET using $HOCH_2CH_2SH$ or GSH as RS• radical precursor,[28] and egg lecithin or SAPC with $HOCH_2CH_2SH$,[46] has been studied in some detail, to understand the PUFA residues behavior. It resulted that the lipid supramolecular organization of vesicles has a profound effect on the isomerization progress for the differentiation of the various double bonds. Trends of the reactivity indicated the overall picture of geometric isomerization in model membranes by the action of diffusible free radicals. The amphiphilic thiyl radical, entering the hydrophobic region of the membrane bilayer, starts to isomerize the double bonds nearest to the glycerol bridge (Figure 5). The sequences of events are driven by both the supramolecular arrangement of the hydrocarbon tails and the highly defined lateral diffusion. An example is given in Figure 4a and b for egg lecithin in solution and LUVET, respectively.[46] The preference of double bonds in positions 5 or 8 of arachidonate residues is followed by position 9 of oleate and linoleate, etc. Figure 4b shows that arachidonate (blue ●) residues are isomerized with more efficiency as compared to oleate (green ●) or linoleate (red ●) residues, and that the isomerization rates of oleate and linoleate were more similar than in solution, where linoleate residues isomerized 2 times faster than oleate (Figure 4a). In particular, the isomerization rate of 9cis of linoleate is very similar to that of oleate (data not shown). For a particular PUFA residue, high regioselectivity is also observed for the mono-trans isomers: in the linoleate residues, the 9trans,-12cis isomer prevailed over the 9cis,12trans isomer, whereas in the arachidonate residues the isomerization occurred in the 5 and 8 positions (cf. Figure 5). These findings are important for monitoring the endogenous formation of trans lipids (see below).

The above-described biomimetic model of thiyl radical-catalyzed isomerization of cis phospholipids in LUVET has recently been used as a sensitive analytical tool based on the amplification of the damaging potential of low-molecular-weight thiols through the catalytic cycle of cis−trans isomerization.[47] In particular, γ-irradiation of DOPC vesicle suspension containing RNase A showed protein degradation with formation of DEPC (trans lipid). Evidence that $CH_3S^{\bullet}$ radicals, deriving from the degradation of methionine residues, are the isomerizing agents has been obtained. Therefore, similar applications of trans

Scheme 6. Enzymatic Fatty Acid Transformations



lipids as markers of radical damage to sulfur-containing proteins can be envisaged in future work.

A Marker of Endogenous Trans Lipid Formation

The distinction between the endogenous formation of trans lipids by free radical attack and dietary supplementation is an open subject, involving the new field of lipidomics, which aims at providing a comprehensive analysis of membrane lipid composition and all lipid pattern and modifications in living organisms. A clue can be obtained from the biomimetic studies in LUVET discussed above, where it was shown that the thiyl radical preferentially attacks the double bonds closest to the glycerol moieties. In this view, arachidonate residues could be proposed as a marker of endogenous trans lipid formation,[46] based on the biosynthetic transformation of linoleic to arachidonic acid shown in Scheme 6. The final four double bonds originate in vivo from different contributions: two enzymatic desaturation steps provide the double bonds in positions 5 and 8, rigorously in the cis configuration, whereas the double bonds in the 11 and 14 positions can be cis and/or trans, depending on the dietary supplementation of natural or chemically modified linoleic acid. Whether this geometrical isomerism might be detected and considered as marker of a radical process are intriguing questions. GC analysis of eicosenoic fatty acid residues obtained after isomerization in model vesicles was similar to that of erythrocyte membranes exposed to a radical stress caused by γ-irradiation.[29,46] Analysis of mono-trans arachidonic acid isomers in human blood plasma led to the conclusion that all four isomers are present, thus confirming the possibility of endogenous and exogenous paths.[48] It should be mentioned that lipids circulating in blood plasma can undergo a random isomerization; therefore, a parallel monitoring of membrane lipids could be recommended for gathering more information on the trans isomer origin. A recent investigation on human monocytic leukemia cell membranes (THP-1) has shown that trans lipids are found before and after treatment with a 10 mM series of thiols, the trans content being higher in the latter case. The similarity of isomer trends formed under incubation and radical stress conditions offers the first evidence that geometrical trans lipids are formed in eukaryotic cells.[49] Further work is in progress for the full characterization and use of other long-chain PUFA residues (e.g., EPA and DHA), in membrane lipids as markers for in vivo radical-induced isomerization. We believe that the radical-catalyzed methodology and the trans lipid library can be important tools for research on lipidomics and recognition of lipid geometry in a biological environment.

Future Perspectives and Biological Consequences

The chemical work done in recent years has pointed out the relevance of cis−trans isomerization by a free radical pathway in the biological context. Generation and reactivity of different thiyl radical species from sulfur-containing biomolecules is a matter of further work, with particular emphasis on competitive processes due to relative reactivity and concentration of substrates in the microenvironment. For example, the detection of trans isomers of arachidonic acid in biological samples has been assigned to the action of $NO_2^{\bullet}$ radicals,[50] although their low reactivity with olefins is known (vide infra), and thiols and urate are the dominant "sink" for $NO_2^{\bullet}$ in cell/tissue and in plasma, respectively.[43] It appears that thiyl radicals are the most harmful species for the cis lipid geometry. This also motivates further studies on isomerization inhibitors, having either molecular or enzymatic modes of action, in connection with the biologically related question on the existence of a surveillance system of lipid cis geometry in cells. Indeed, work on the biological impact of lipid isomerization is still at the beginning. The biological effects of trans lipids, which are clear for a dietary supplementation, are not yet understood for a radical-based endogenous process. In particular, information of the relevant cellular districts involved in this transformation has to be gathered, as well as data about the type and quantity of trans isomers that influence membrane and cell functions. The study of trans lipid formation, and their structural and metabolic roles, represents an interdisciplinary topic, which is expected to advance the knowledge on the meaning of lipid geometry in the biological environment and relate it to health and disease.

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Note Added after ASAP Publication

Multiple revisions were made to Scheme 5, and ref 49 was updated. This paper was originally posted on the Web on March 9, 2005 and again on April 28 and May 11, 2005. The final version was reposted on the Web on May 17, 2005.

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