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Figure 1

218x242mm (240 x 240 DPI)

Figure 2

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Figure 3

213x149mm (240 x 240 DPI)

Figure 4

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Figure 6

180x147mm (240 x 240 DPI)

Figure 7

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Subtle Changes in Lipid Environment Have Profound Effects on Membrane Oxidation Chemistry

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ABSTRACT: Nature carefully designs the components of amphiphile-composed monolayer and bilayer membranes to deliver specific functions. The compositions of these interfacial layered structures are so delicate that minute modifications can result in huge changes in function. Great efforts have been expended to understand membrane physical properties, with only minimum attention given to associated chemical properties. Here we report the first examples of the delicate chemistry associated with membrane amphiphilic components by studying OH mediated oxidation of six different unsaturated lipids/surfactants and their mixtures at the air-water interface using field-induced droplet ionization mass spectrometry (FIDI-MS). When the packing is loose or perturbed to be loose by other components or prior chemical modification, the double bond is oxidized without cleavage by adding oxygen functionality. In contrast, compact packing results in double bond cleavage through a Criegee intermediate mechanism. We postulate that constrained environments imposed by lipid packing limit the conformations of the reaction intermediates, controlling reaction pathways.

INTRODUCTION

Monolayer and bilayer structures comprising amphiphilic molecules occur extensively *in vivo* in the form of various membranes, in the environment as coating of atmospheric aerosols and natural waters, and in condensed phase as dissolved micelles and liposomes. Great efforts are exerted to examine the effect that small structural variations of the molecular components of these interfacial layers have on their physical properties.¹⁻¹² These physical properties include but are not limited to packing density, curvature, surface pressure, fluidity, and permeability, where a higher packing density can usually result in higher surface pressure, lower fluidity, and lower permeability. An important example is the outermost layer of the eye, known as the tear film. It comprises a complex mixture of lipids residing at the air-water interface, preventing the water layer beneath it from evaporating too fast.³ However, the lipid components of dry eye syndrome patients are only slightly different from normal,³ providing a testament to their delicate selection by nature. Another example is that cholesterol acts as a bidirectional regulator in cell membranes, where it can increase the packing density and fluidity at the same time by forming tight cholesterol-lipid assemblies, known as lipid rafts.⁴⁻⁶ Even though the packing density of amphiphilic molecules can be affected by environmental variables such as pressure and temperature, this property is determined by the structures of the lipids or surfactants and their relative ratios. Tanford⁷ suggests that the head group is the major determinant of packing. A larger hydrophilic head group with more permanent charges usually results in higher packing densities when compared to a smaller head group with fewer or without permanent charges.^{2,7} The former can form more ionic or hydrogen bonding attractions with each other and water, resulting in tight packing. In

addition, surfactant chains of lipids with unsaturated C=C double bonds are often more loosely packed than those with saturated chains, because the *cis*-olefin linkage introduces kinks into the otherwise straightened saturated chains.^{2, 8-11} Therefore, packing defects are often associated with lipids and surfactants with small uncharged head groups and unsaturated chains.

While the well characterized physical properties discussed above are indeed critical to determine and maintain the integrity and functions of the monolayer- or bilayer-formed structures, the impact that membrane structure has on associated chemical properties are much less studied or appreciated. This is especially important for biological systems because it is finely tuned biochemical functions that impact health. For example, comprising 30 mol % of the lipids in cell membranes, cholesterol serves the biophysical roles of increasing lipid-packing density and maintaining high membrane fluidity. Our group recently demonstrated that this has important biochemical consequences whereby cholesterol plays a non-sacrificial chemoprotective role in lipid monolayers by inhibiting chemical damage by potent reactants such as hydroxyl radical (OH) and HCl.¹³

Given that investigations addressing the impact of surfactant/lipid composition and packing on membrane chemistry are long overdue, we report herein the OH mediated oxidation of six different amphiphilic molecules and several of their mixtures at the air-water interface using the home-developed field-induced droplet ionization mass spectrometry (FIDI-MS) methodology. This technique is capable of selective “online” sampling of molecules that reside at the air-water interface. These six molecules include oleylamine (OAm), oleic acid (OAc), 1-palmitoyl-2-oleoyl-sn-glycerol

(PODAG), 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (POPG), 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (SOPC), and 1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DOPG). All of them have the same reactive functional groups towards OH oxidation, namely at least one unsaturated C=C double bond on an oleyl chain and many saturated C-H bonds. The OH radical is anticipated to exhibit significantly higher reactivity with the C=C bond compared to the saturated C-H bonds, since the rate coefficients for oxidation of unsaturated hydrocarbons by OH are significantly higher than corresponding saturated molecules.¹⁴ These six molecules have very distinct structures (Figure S1): OAm and OAc have one unsaturated chain and small head groups, PODAG has two chains (one unsaturated) with a small head group that does not possess permanent charge, POPG and SOPC have two chains (one unsaturated) with large head groups that have permanent charges, and DOPG has two unsaturated chains with a large head group that has a permanent charge. Hence, it can be expected that OAm, OAc, and PODAG have looser packing, and POPG, SOPC and DOPG have denser packing. Their relative packing densities have been previously reported:¹⁵⁻¹⁸ the area per lipid (APL) of POPG, SOPC and DOPG ranges from 63 to 70 Å²/lipid (31 to 35 Å²/chain), the APL of PODAG is at least 85 Å²/lipid (42.5 Å²/chain), and the APLs of OAm and OAc are also around 42 Å²/chain. So the packing densities of POPG, SOPC and DOPG are indeed higher than those of PODAG, OAm and OAc.

We study monolayers of lipids and surfactants as well as their mixtures at the air-water interface because they are ubiquitous in nature, present as the coating of natural waters,¹⁹ comprising half of bilayer cell membranes, and the exposed surface of pulmonary alveoli²⁰ as well as the tear film³ that are directly in contact with air. Hence, amphiphilic monolayers are often the frontier that engages destructive chemical processes. OH is chosen in this study because it is a potent oxidant that exists both in the atmosphere and *in vivo*.²¹⁻²³ Once inhaled or generated *in vivo*, it can rapidly attack virtually all molecules.

EXPERIMENTAL SECTION

PODAG, POPG, SOPC, and DOPG were purchased from Avanti Polar Lipids (Alabaster, AL, USA) and used without further purification. OAm was purchased from Sigma Aldrich (St. Louis, MO, USA) and used without further purification. All solvents (water and methanol) are HPLC grade and purchased from EMD Chemicals Inc. (Gibbstown, NJ, USA). 2 mM stock solutions of these five molecules are firstly obtained by dissolving the chemicals in methanol, and then diluted by water to make solutions of 100 μM pure OAm, PODAG, POPG, SOPC, DOPG, 100 μM POPG/20 μM OAm mixture, 100 μM POPG/100 μM OAm mixture, 33 μM POPG/100 μM OAm mixture, and 100 μM DOPG/100 μM OAm mixture solutions.

Figure S2 presents the schematic drawing of the field-induced droplet ionization mass spectrometry (FIDI-MS) setup. The FIDI-MS methodology has been proven to be interfacially sensitive, and it selectively samples molecules that are at the air-water interface.^{24, 25} Briefly, a hanging droplet of ~2 mm o.d. (~4 μL in volume) is suspended on the end of a stainless steel capillary between two parallel plate electrodes separated by 6.3 mm. Droplets are formed from liquid fed through the capillary using a motorized syringe pump. The parallel plates are mounted on a translation stage to

allow alignment of an aperture in the electrically grounded plate with the atmospheric pressure inlet of an LTQ-XL mass spectrometer (Thermo-Fisher, Waltham, MA). The capillary is mounted on a separate translation stage to place the droplet exactly midway between the two plates and to align with the inlet of the LTQ-XL. Mass spectrometric sampling of the hanging droplet is accomplished by applying a pulsed high voltage (typically 3 to 5 kV, 5 ms duration, polarity selected to sample either positive or negative ions) to the back parallel plate and to the capillary at half the magnitude applied to the back plate to maintain field homogeneity between the front and back plate. When a sufficiently high voltage is applied, bipolar ejection of highly-charged progeny droplets less than 1 μm in diameter from the opposite ends of the suspended droplet ensues. Charged droplets of a selected polarity enter the transfer capillary of the mass spectrometer, resulting in the detection of gas-phase ions. In this study, we apply positive voltage on the back plate in order to detect the protonated OAm, sodiated PODAG, sodiated or protonated SOPC cations and their oxidation products, and negative voltage to detect desodiated POPG, DOPG and their oxidation products. When each droplet is initially formed, we allow 60 seconds for the molecules to diffuse to the air-water interface before exposing the droplet to hydroxyl radicals for a variable reaction time. After the reactions, we trigger the high voltage to sample both reactants and products. Sampling occurs on a millisecond time scale. Each experiment starts with a fresh drop.

Hydroxyl radicals are generated using a dielectric barrier discharge source (DBDS) composed of a borosilicate tube (1/4" OD, 3/16" ID) which acts as the dielectric material. A tungsten filament inner electrode is sealed within the tube, and a conductive silver epoxy coating (McMaster-Carr, Santa Fe Springs, CA, USA) acts as an outer electrode. A glass bubbler provides water saturated helium through the DBDS, with a flow of 1000 mL/min monitored by a Type π MFC Digital Mass Flow Controller (Model PFC-50, MKS Instruments). A high voltage AC power supply (Trek PM04015) biased the inner electrode during experiments at 12 kV (peak to peak) and 1000 Hz, while the outer electrode remained grounded. Between the power supply and the tungsten filament, there is a 1 MΩ resistor used as a current limiter. A low temperature plasma (dielectric barrier discharge) is generated inside the tube, producing hydroxyl radicals in the gas flow. Compared to the tropospheric OH radical concentration that was measured to be $1-3 \times 10^6$ molecules cm⁻³,²¹ the DBDS can generate $\sim 1 \times 10^9$ molecules cm⁻³ OH radicals²⁶ based on the time required to oxidize a monolayer of surfactant with an average of one oxygen per surfactant molecule. The high intensity of OH radicals significantly accelerates the oxidation process compared to ambient conditions.

Results and Discussion

Figure 1 presents the time-resolved oxidation of pure OAm and PODAG monolayers for various exposure time periods to OH radicals at a density of approximately 10^9 cm⁻³ in air. The product distributions in these two cases have the same pattern. The m/z 268 peak in Figure 1a is the protonated OAm parent cation, and the m/z 617 in Figure 1b is the sodiated PODAG parent cation. The peaks with higher m/z are the oxidation products. In both cases, the oxidation proceeds by a pattern of adding 16 Da (m/z 284 or 633), 14 Da (m/z 298 or 647), and then 16 Da (m/z 314 or 663). These m/z 284 or 633 products are initiated by the addition of OH to the C=C bond, followed

by steps mediated in part by ambient O_2 molecules, forming carbonyl functional groups. The other higher m/z products are results of oxidation of saturated C-H bonds originated by abstraction of hydrogen atoms from C-H bonds by OH radicals, followed by steps mediated by ambient O_2 and NO molecules, forming carbonyl and hydroxyl functional groups (*vide infra*). Since the major products are the addition of oxygen functionality to the parent molecules, we name this process “addition chemistry”. At the lower m/z side, both molecules have minor products at m/z 158 or 507, resulting from the C=C bond being cleaved into an aldehyde group. At 45 s or 40 s, almost all of the OAm and PODAG are oxidized with the addition of one or more oxygen atoms. The OH oxidation of OAc has been previously reported by our group,²⁶ and the oxidation pattern is similar to OAm and PODAG. The similarity among these three molecules is comprehensible in the sense that they have the same functional groups subject to OH oxidation, and they are all relatively loosely packed.

Figure 1. Time-resolved FIDI-MS spectra of the OH oxidation reactants and products of two surfactants at the air-water interface. (a) Protonated OAm and its oxidation products appear at 268 and higher m/z . Low abundance bond cleavage products are observed starting at m/z 168. (b) Sodiated PODAG and its oxidation products appear at 617 and higher m/z . Low abundance bond cleavage products are observed starting at m/z 507.

Figure 2 shows the OH oxidation of POPG and SOPC for 60 s. The time-resolved study of POPG has been previously reported,¹³ so here we only present a representative spectrum. The m/z 747 peak in Figure 2a is the desodiated POPG parent anion and the m/z 789 and 811 peaks in Figure 2b are the protonated and sodiated SOPC parent cations. Surprisingly, in complete contrast to OAm and PODAG, the oxidation products of POPG and SOPC appear at lower m/z where the chemistry is dominated by the highly reactive double bond on the oleoyl chains. The m/z 637 product of POPG and the m/z 679 and 701 products of SOPC are results of the double bond cleaved into aldehyde groups. The m/z 671 peak of POPG and the m/z 713 and 735 peaks are from the oxidation of the

double bond into an hydroxyhydroperoxide (HHP) molecule via a Criegee intermediate (CI) (*vide infra*). We call the process “CI chemistry”. Noteworthy is the fact that CI is an important atmospheric radical resulting from the reaction of ozone with olefins both in the gas phase²⁷ and at the air-water interface,²⁸ and a major contributor to OH radicals in the troposphere.²⁹ Our results provide evidence that OH oxidation of tightly packed unsaturated hydrocarbons can also generate CI.

Figure 2. FIDI-MS spectra of the OH oxidation reactants and products of two phospholipids after 60 s of oxidation. (a) Desodiated POPG and its oxidation products appear at 747 and lower m/z . (b) Sodiated/protonated SOPC and its oxidation products appear at 811/789 and lower m/z .

Since the above discussed molecules all have the same oleoyl chain to react with OH, and PODAG, POPG and SOPC have the spectator saturated hydrocarbon chains nearby, the striking difference between the addition chemistry of OAm, OAc and PODAG, and CI chemistry of POPG and SOPC must be attributable to the different packing density. In Figure 3a, we introduce the results of DOPG, a lipid with a large head group and two unsaturated chains. The m/z 773 peak is the desodiated parent anion. The major products appear at lower m/z . The m/z 663 peak is the double bond cleaved into an aldehyde and the m/z 697 peak is the HHP product, this being the same CI chemistry as observed for POPG and SOPC. However, after the first double bond is cleaved, the second double bond does not cleave into even lower m/z products. Instead, it follows the pattern for addition chemistry, resulting in the m/z 679 (16 Da more than m/z 663) and the m/z 713 (16 Da more than 697) products. This result further confirms the suggestion that packing density determines the chemistry. Figure 3b depicts this process: after losing the company of the first chain that is cleaved through the CI chemistry, the second chain becomes loosely packed, leaving it to follow the addition chemistry reaction path. The fact that the products

from CI chemistry (m/z 663 and 697) have similar intensity as the products from the addition chemistry (m/z 679 and 713) also agrees with the suggested reaction sequence with CI chemistry occurring first, followed by the addition chemistry. The oxidation chemistries of the above discussed six molecules are tabulated in Table 1 along with their relative packing densities.

Figure 3. FIDI-MS spectra of the OH oxidation reactants and products of DOPG after 60 s of oxidation. (a) Desodiated DOPG and its oxidation products appear at 773 and lower m/z . Low abundance products are observed at m/z higher than 773. (b) A cartoon showing the packing change of the second chain of DOPG after the chain is cleaved.

Table 1. Areas/chain for a measure of packing density and the oxidation chemistries of the six molecules studied.

Molecule	Packing density ($\text{\AA}^2/\text{chain}$)	Chemistry
PODAG	$>42.5^{16}$	Addition
OAc	42.2^{15}	Addition
OAm	40.0^{15}	Addition
DOPG	34.7^{17}	Mixed
POPG	32.2^{17}	CI
SOPC	31.9^{18}	CI

To further test the idea that packing density changes the chemistry, mixtures of OAm with POPG, and OAm with DOPG were studied to see if OAm could perturb the packing of POPG and DOPG. In Figure 4a, results from the 60 s oxidation of pure POPG, POPG/OAm = 5/1, 1/1 and 1/3 are presented. When POPG/OAm = 5/1, a mixed chemistry is observed with lower m/z products from the CI chemistry and higher m/z products at 763 and 777 from the addition chemistry, indicating that POPG packing is already loosened by the addition of only 16.7% OAm. This suggests a very high sensitivity of the chemistry towards the composition of the monolayer. When POPG/OAm = 1/1 and 1/3, the CI chemistry is fully inhibited, and only the addition chemistry is observed. The product intensity is higher in the 1/3 case than the 1/1 case, suggesting that higher OAm ratio loosens the monolayer more, giving OH higher permeability into the interface. As is clearly evident in Figure 4b, the 1/1 mixture of DOPG and OAm also fully transforms the CI chemistry into the addition chemistry after 30 s of oxidation.

When the area per lipid is $60 \text{ \AA}^2$, the minimum concentration to form a monolayer lipid at the air-water interface of a 2 mm o.d. droplet is around $8 \text{ \mu M}$, so the $100 \text{ \mu M}$ concentration used in the above experiments is enough to form a monolayer. Since we cannot manually compress the monolayers of OAc or OAm using the current setup, we studied the OH oxidation chemistry of a $1 \text{ \mu M}$ solution of POPG to guarantee a loosely packed monolayer (Figure S3). Addition chemistry is observed, which further supports our conclusion that packing density is a key factor determining the oxidation chemistry. Not unexpectedly, as a result of the relatively low concentration, the signal/noise obviously suffers in Figure S3.

The product yields from OH oxidation of POPG, SOPC and DOPG are much lower than those of OAm and PODAG, even after longer exposure times. It is understandable that tight packing can lower the oxidation rate by lowering the OH radical permeability, similar to our earlier reported results for the markedly reduced oxidation of monolayers of lipids mixed with 30 mol % cholesterol.¹³ This observation provokes the obvious question as to why does the packing density, a physical property, give completely different chemistries in these seemingly similar reactive systems.

Figure 4. FIDI-MS spectra of the OH oxidation reactants and products of OAm and phospholipid mixtures. (a) Gradual change of oxidation chemistry of POPG when different amounts of OAm is added to the monolayer. (b) The addition chemistry of DOPG studied in the OAm/POPG mixture.

To answer this question, we first discuss the reaction mechanisms. Figure 5a exhibits the proposed mechanisms of the addition chemistry. It starts with a C=C double bond belonging to OAm, OAc, PODAG, the second C=C bond in DOPG, or the perturbed POPG and DOPG. Step (1) is the uptake of one OH radical from the gas phase by either carbon atom on the double bond, forming a β -hydroxyalkyl radical. This OH addition step is the well-accepted first reaction of OH and alkenes.^{13, 30} Afterwards, the hydroxyalkyl radical uptakes an O_2 molecule to form a hydroperoxy radical in step (2) since the concentration of ambient O_2 is many orders of magnitude higher than the OH used in the current study ($\sim 1 \times 10^9 \text{ molecules cm}^{-3}$). The peroxy moiety could abstract an

Figure 5b presents the proposed mechanism of the CI chemistry of POPG, SOPC and the first C=C bond of DOPG. Step (1) and (2) are the same as the addition chemistry, forming two different β -hydroxyperoxy radicals, one of which can undergo β -cleavage to an α -hydroxyalkyl radical (step (3)), and the latter can further react with O_2 to form an HO_2 leaving group and an aldehyde molecule, corresponding to m/z 637 for POPG (Figure 2a), 679 or 701 for SOPC (Figure 2b), and 663 for DOPG (Figure 3a). The aldehyde reacts with H_2O to form the minor hemiacetal product (m/z 655 for POPG and 681 for DOPG). The second β -hydroxyperoxy radical formed in step (2) can also undergo thermal dissociation into a Criegee intermediate (CI) and a hydroxyalkyl radical (step (6)). This CI product, written in its favored zwitterionic form, can react with the CI scavenger, H_2O , to form the hydroxyhydroperoxide (HHP) product (step (7)), corresponding to m/z 671 for POPG (Figure 2a), 713 or 735 for SOPC (Figure 2b), and 697 for DOPG (Figure 3a). The HHP product is a symbolic evidence of the CI reaction pathway³⁶ and eventually becomes an abundant product. This mechanism for generation of CI from reaction of OH with olefins was first proposed in our previously reported reaction of POPG and OH,¹³ where expected products from several CI scavengers³⁷ were observed, and *ab initio* calculations were performed to justify the proposed mechanism.

To explore the energetics associated with the CI and addition chemistries, potential energy surface calculations using C_2H_4 as a model system are presented in Figure 7. The black lines denote the CI chemistry pathway that is similar to that of Reference 13, and the red lines denote the addition chemistry pathway. All of the structures are optimized at the $\omega\text{B97X-D/6-311++G(3df, 3pd)}$ level of theory with zero-point energy correction using the Gaussian09 package. Without constraints from the environment, the products formed in addition chemistry should have lower energy than its CI counterpart. It is indeed so as shown by Figure 7. Even the transition state (TS) of the enol-forming step along the addition chemistry pathway has lower energy than the CI-forming step along the CI chemistry pathway, manifesting that addition chemistry is thermodynamically favored if it is not sterically hindered.

Figure 6. How difference in packing density changes the oxidation chemistry of the monolayer.

Conclusion

The OH oxidation chemistry of the monolayers formed by six different amphiphilic molecules and their mixtures at the air-water interface has been studied using the FIDI-MS methodology. The oxidation chemistry is delicately influenced by the packing densities of the hydrophobic appendages. The chemistry of relatively loosely packed OAm, OAc, PODAG, the second C=C bond of DOPG, POPG/OAm mixture, and DOPG/OAm mixture complies with a patterned addition chemistry, where the C=C double bonds are first oxidized into carbonyl groups, followed by the C-H saturated bonds being oxidized to form additional carbonyl and hydroxyl groups. The addition chemistry pathway goes through a hydroxyperoxy radical, where the loosely packed environment allows eclipsed conformations so that the O_2 moiety can contact the adjacent H atom to eliminate HO_2 (Figure 5a and Figure 6). The oxidation chemistry of relatively densely packed POPG, SOPC and the first C=C bond of DOPG conforms to the CI chemistry. The C=C double bond is cleaved into an aldehyde molecule and an HHP molecule. In a similar hydroxyperoxy radical, only staggered conformations are allowed due to the densely packed environment (Figure 6), where the O_2 moiety cannot contact the adjacent H atom, constraining the elimination of HO_2 . Instead, the chemistry can only occur along the 1D hydrocarbon chain, resulting in the C-C bond cleavage.

For the first time the delicacy of the compositions of the surfactant/lipid composed monolayers has been revealed to have profound influence on the chemical reaction pathways. The OH oxidation chemistry of the C=C double bond is a noteworthy chemomarker of the packing density of amphiphilic molecular layers.

Figure 7. Reaction potential energy surfaces (eV) for the CI and addition chemistries using ethylene as a model system at the ω B97X-D/6-311++G(3df, 3pd) level of theory.

ASSOCIATED CONTENT

Supporting Information

Additional distinct structures of six molecules and the schematic drawing of the FIDI-MS setup. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no completing financial interest.

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