Interplay between Structure and Fluidity of Model Lipid Membranes under Oxidative Attack

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A proper regulation of membrane fluidity is critical for cellular activities such as communication between cells, mitosis, and endocytosis. Unsaturated lipids, a main component of biological membranes, are particularly susceptible to oxidative attack of reactive oxygen species. The oxidation of lipids can produce structural derangement of membranes and eventually alter the membrane fluidity. We have applied fluorescence correlation spectroscopy (FCS) and Raman spectroscopy to investigate the fluidity and structure of model membranes subject to oxidative attack. Hydrogen peroxide has little effect on the lateral fluidity of membranes, whereas hydroxyl radical causes a significantly increased fluidity. The latter is rationalized with the cleavage of the acyl chains of lipids caused by hydroxyl radical; this interpretation is founded on the diminished intensities of lines in Raman spectra associated with −CH2 and C=CH moieties in lipids and supported by mass-spectral measurements. The same approach provides a mechanistic account of the inhibitory capability of vitamins C and E against the increased membrane fluidity resulting from an oxidative attack. Membranes with much cholesterol exhibit a novel resistance against altered membrane fluidity induced with oxidative attack; this finding has biological implications. Our approach combining FCS and Raman measurements reveals the interplay between the structure and fluidity of membranes and provides insight into the pathophysiology of cellular oxidative injury.

Introduction

The regulation of membrane fluidity, especially the lateral diffusion of molecules on biological membranes, is essential for cells to maintain vital activities such as cell motility, mitosis, recognition, endocytosis, and phagocytosis.1–10 An altered membrane fluidity has been observed to occur concurrently with, or has been implicated in, various diseases.11–16 The fluidity of biological membranes is controlled mainly by their compositions.17,18 Membranes made of lipids with short acyl chains, or with many unsaturated C=CH moieties in the fatty chain, tend to have great fluidity.18 As a result, an external stress that causes structural modification of the constituent lipids of membranes would yield also an altered membrane fluidity. Through their reactivity, unsaturated lipids, a main component of biological membranes, are susceptible to the oxidative attack of endogenous reactive oxygen species (ROS), such as hydrogen peroxide (H2O2) and hydroxyl radical (OH•).20,21 Despite considerable effort to observe the modification of membrane fluidity induced by oxidative attack, few provide mechanistic or structural account to this alteration. Among contradictory conclusions reported, increased fluidity was observed for brain membranes subject to oxidative attack. Hydrogen peroxide has little effect on the lateral fluidity of membranes, whereas hydroxyl radical causes a significantly increased fluidity. The latter is rationalized with the cleavage of the acyl chains of lipids caused by hydroxyl radical; this interpretation is founded on the diminished intensities of lines in Raman spectra associated with −CH2 and C=CH moieties in lipids and supported by mass-spectral measurements. The same approach provides a mechanistic account of the inhibitory capability of vitamins C and E against the increased membrane fluidity resulting from an oxidative attack. Membranes with much cholesterol exhibit a novel resistance against altered membrane fluidity induced with oxidative attack; this finding has biological implications. Our approach combining FCS and Raman measurements reveals the interplay between the structure and fluidity of membranes and provides insight into the pathophysiology of cellular oxidative injury.

Being principal constituent of the membranes of both mammalian cells and intracellular organelles, cholesterol has maintained intense interest.26–28 It not only regulates the bulk physical properties of membranes, but also engages in specific interaction with other membrane constituents such as proteins. The cholesterol content of membranes varies significantly among cell types or organelles;29,30 for example, the plasma membrane of human erythrocytes contains approximately 45 mol % cholesterol with respect to other membrane lipids, whereas the membrane of intracellular organelles such as mitochondria and endoplasmic reticulum contains less than 10 mol %.31 Although the modulation of membrane fluidity by cholesterol has been investigated,18,19 whether cholesterol specifically affects the ROS-induced alteration of membrane fluidity has barely been addressed. In view of the large variation of cholesterol content among cells or organelles, an answer to the above question should provide clues to the fates of various cells or organelles under oxidative attack.

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To investigate the interplay between the structure and the fluidity of membranes under oxidative attack, we employed a parallel approach based on fluorescence correlation spectroscopy (FCS)\textsuperscript{32–35} to characterize the lateral diffusion of molecules on membranes, and Raman spectroscopy,\textsuperscript{36–38} to characterize the structure of lipid molecules of single optically trapped liposomes. We compared the alteration of membrane fluidity under treatment with two ROS, hydrogen peroxide and hydroxyl radical, and examined the ability of vitamins C and E to inhibit this change. We show that Raman spectra provide structural information to account for the fluidity change. In our investigation of membranes comprising varied cholesterol content, we discovered a novel inhibitory effect of membrane-bound cholesterol against ROS-induced alteration of membrane fluidity. The results are rationalized with complementary Raman spectra. Our result demonstrates the unique capability of this parallel approach and indicates its potential to enhance our understanding of cellular oxidative injury.

Materials and Methods

**Reagents.** \textsuperscript{1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC, Avanti Polar Lipids), 1,1′-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine perchlorate (DiD), 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO) and Alexa Flour 633 (Invitrogen), trichloromethane and menthol (J.T. Baker), cholesterol, glucose, sodium chloride (NaCl), hydrogen peroxide, FeSO\textsubscript{4}·7H\textsubscript{2}O, L-ascorbic acid and α-tocopherol (Sigma Aldrich) were obtained from the indicated sources.**

Hydroxyl radical was prepared on mixing equal amounts of solutions of H\textsubscript{2}O\textsubscript{2} and FeSO\textsubscript{4}; its concentration was estimated from the indicated sources.\textsuperscript{38}

Vitamin C solution was prepared on dissolving L-ascorbic acid in deionized water and was mixed with a solution containing lipid vesicles prior to measurements.

**Preparation of Giant Unilaminar Vesicles (GUV).** We employed electroformation to prepare GUV according to a reported protocol.\textsuperscript{39} In brief, we prepared an appropriate mixture of lipid and cholesterol in a solution containing trichloromethane and methanol (volume ratio 9:1), transferred the solution (20 µL) to a glass slide coated with indium tin oxide (ITO), and kept the slide under vacuum for at least 1 h to evaporate the solvent. We then placed a silicone spacer on the slide, added a solution of glucose (600 µM, 0.2 M) to the slide, and sealed the solution with another ITO-coated glass slide. To produce vesicles, we supplied an alternating bias (sinusoidal waveform, \(\tau_0 = 3 \text{ V}, f = 10 \text{ Hz}\)) to the two conducting slides for 3 h. All subsequent measurements were performed at 22 ± 1 °C. To prepare GUV containing vitamin E, the same protocol used to prepare DOPC GUV was followed except that a small amount of vitamin E was mixed with DOPC (molar ratio 1:20) in the solution.

For confocal fluorescence imaging and FCS measurements, we used two fluorescent probes specific to lipids, DiO and DiD, respectively, to label vesicles. The preparation of the fluorescence-labeled vesicles was identical to that of nonlabeled vesicles, except that the labeling agents (molar ratios 10\textsuperscript{-8} for confocal fluorescence imaging and 10\textsuperscript{-5} for FCS measurements) were mixed in a small portion with lipids in the solution. The diameter of vesicles prepared in this way ranged mostly between 20 and 30 µm, as can be seen from the confocal images displayed in Figure 1.

**FCS Measurements.** A schematic of our constructed FCS system used in this work is shown in Figure 2A. The beam of the HeNe laser (1137P, JDS Uniphase, U.S.A.) for excitation was expanded and collimated to a diameter about 1 cm with a spatial filter. The collimated beam was directed to an inverted optical microscope (IX 71, Olympus, Japan) through the backport using a dichroic mirror and was focused onto the sample with a microscope objective (60× PlanApo, water immersion, numerical aperture 1.2, Olympus, Japan). The typical laser power illuminating the sample was 38 µW. A three-axis piezo stage (PI-563CD, Physik Instrumente, Germany) integrated into the sample stage of the microscope served to position the vesicles with respect to the laser focus. The emitted fluorescence was collected in an epi direction, passed through the dichroic mirror and a bandpass filter, and detected with a fiber-coupled avalanche photodiode (APD, SPCM-AQR-15-FC; PerkinElmer Optoelectronic, Canada). The optical fiber (core diameter 50 µm) acted as a pinhole to achieve confocal detection. The autocorrelation function of the fluctuating fluorescence signal was calculated in real time with a hardware correlator (Correlator.com, U.S.A.).

To derive the diffusion coefficient of DiD molecules on membranes, we fitted the autocorrelation function with an equation based on a model of two-dimensional diffusion\textsuperscript{34,40,41}

\[
G(\tau) = \frac{1}{N} \left[ 1 + \frac{\tau}{\tau_D} \right]^{-1}
\]

in which \(G(\tau)\) is the autocorrelation function, \(N\) is the effective number of DiD molecules within the detection zone, \(\tau\) is the delay time and \(\tau_D\) is the diffusion time. Data fitting was performed with an algorithm based on least-squares. After the diffusion time was obtained from the best fit, the diffusion coefficient, \(D\), was calculated from

\[
D = \frac{r_0^2}{4\tau_D}
\]

in which \(r_0\) is the waist (1/e\textsuperscript{2} radius) of the focal zone, is a system parameter that was determined independently using a reference molecule of known diffusion coefficient in solution. Figure 2B shows an autocorrelation curve obtained from a reference (Alexa 633) in solution. Using a diffusion coefficient \(D = 1.35 \times 10^{-10}\) m\textsuperscript{2} s\textsuperscript{-1}, we determined the waist of the focal zone to be 203 nm. For comparison, a representative autocorrelation curve measured from DiD molecules on membranes is included.
We measured only vesicles of similar size (diameter $\sim 25 \mu m$). The large size relative to the dimension of the detection zone ensured that the curvature of the membrane had a negligible effect. For consistency, all FCS measurements were performed near the center of the upper membrane of vesicles. We first located the center of an arbitrary focal plan within a vesicle and then translated the vesicle in the vertical direction to obtain a maximal intensity of fluorescence. Representative cross-section intensities of lateral and vertical scans are shown in Figure 2C. We estimated that the lateral and vertical resolutions of our apparatus were 0.3 and 1.1 $\mu m$, respectively, from the full width at half-maximum of these line scans. To improve the alignment of the laser focus with the upper membrane, we measured depth-resolved FCS with step size 0.2 $\mu m$. Figure 2D shows the diffusion coefficient and the effective number of molecules at varied depths near the upper surface of a vesicle. Subsequent FCS measurements were made at the depth that yielded the largest diffusion coefficient and the smallest number of fluorescent molecules. Typically, 10 successive measurements (duration 10 s) were performed for each vesicle to obtain a statistical mean.

**Raman Spectral Measurements on Single Liposomes.** We employed “Raman tweezers” to measure temporally varying Raman spectra of single optically trapped liposomes and to characterize kinetically the structural modification of lipid molecules under treatment with ROS; details of this apparatus are reported elsewhere. In brief, a diode-pumped solid-state laser (532 nm, DPSS Inc., U.S.A.) served both to capture a single lipid liposome and to generate Raman scattering from the optically trapped liposome. The backscattered Raman signal was recorded with a spectrometer (SpectraPro 2300, Princeton Instruments, U.S.A.). We continuously recorded the spectra of a single liposome for as long as 60 min with an acquisition duration 60 s for each spectrum. From each spectrum we subtracted the background; the serial changes of each line were expressed as the fold of change relative to the baseline spectrum obtained in the first minute. The spectral resolution of the system, 1.6 cm$^{-1}$, was determined from the full width at half-maximum of the line of polystyrene at 1001 cm$^{-1}$.

**Statistical Methods.** Comparison between the means of two groups was made using the two-tailed Student’s t test. The levels of statistical significance were set at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

**Results and Discussion**

**Oxidative Attack Causes an Increase of Membrane Fluidity through Cleavage of the Acyl Chain of Lipids.** We sought first to determine how a treatment with ROS altered the membrane fluidity. Figure 3A displays representative autocorrelation curves of DiD molecules on DOPC membranes obtained before and after exposure (60 min) to hydroxyl radical (0.05 mM); results obtained under treatment with hydrogen peroxide (0.05 mM) are shown in Figure 3B. A comparison of these results shows that the treatment with hydroxyl radical caused the autocorrelation curve to shift to the left, whereas treatment with hydrogen peroxide yielded no discernible alteration of the autocorrelation curve.

The diffusion coefficients of DiD molecules obtained on membranes subject to the two treatments are shown in Figure 3C; the diffusion coefficient increased by 62% (6.52 $\times 10^{-8}$ vs $10.59 \times 10^{-8}$ cm$^2$ s$^{-1}$, $P = 5.87 \times 10^{-8}$) when the membrane...
was treated with hydroxyl radical for 60 min, whereas the change was insignificant with the treatment of hydrogen peroxide (6.52 × 10⁻⁸ vs 6.49 × 10⁻⁸ cm² s⁻¹, P = 0.88). This finding clearly shows that the exposure of the membranes to hydroxyl radical produced a greatly increased membrane fluidity, whereas hydrogen peroxide, if not converted to hydroxyl radical, had little effect on the membrane fluidity.

To provide a structural account of the ROS-induced alteration of membrane fluidity, we employed kinetic Raman measurements to characterize the spectral variation of liposomes comprising DOPC lipids under treatment with hydroxyl radical or hydrogen peroxide; the results are displayed in Figure 4. To facilitate a comparison of the progressive spectral changes, we highlighted the two most pronounced Raman lines, at 1440 cm⁻¹ attributed to the CH₂-bending mode (light gray) and at 1650 cm⁻¹ attributed to the C=C-stretching mode (dark gray). As seen from Figure 4A, the sequential Raman spectra of the control (i.e., with no treatment) exhibited no appreciable temporal variation (>60 min) confirming that a prolonged exposure of DOPC liposomes to laser illumination incurred no discernible damage under our experimental condition. When the liposome was treated with hydroxyl radical (0.05 mM), both specified Raman lines diminished progressively (Figure 4B). In contrast, the sequential Raman spectra of a single liposome on treatment with hydrogen peroxide (0.05 mM) remained essentially constant (Figure 4C).

The decreased intensity of the Raman line at 1650 cm⁻¹ (C=C stretching mode) offers a sensitive means to probe the progression of lipid peroxidation in both biological cells and liposomes comprising monounsaturated lipids. This work confirms that conclusion by showing that a treatment with hydroxyl radical induced peroxidation of DOPC lipids. It also shows that, in contrast to hydroxyl radical, hydrogen peroxide is insufficiently reactive to cause discernible structural modification.

Figures 4 and 3 clearly show that the variation of the Raman spectra exhibited a trend consistent with that of the diffusion coefficients according to the results of treatments with hydroxyl radical and hydrogen peroxide. As Raman spectra provide structural information, this observation indicates a strong correlation between the alteration of the membrane fluidity and the modification of the lipid structures. We sought to account for the observed increase of membrane fluidity by solving the structural clues of Raman spectra. We analyzed the sequential variation of the Raman spectra of single optically trapped liposomes subject to the treatment with hydroxyl radical (n = 10); the temporally varying intensities of the Raman lines at 1440 and 1650 cm⁻¹ are displayed in Figure 4D. After exposure (60 min) to hydroxyl radical, the intensities of both Raman lines decreased, by 20% (light gray, P = 8.39 × 10⁻⁵) and 30% (dark gray, P = 1.17 × 10⁻⁴), respectively. As the intensities of the two specified Raman lines are proportional to the number of CH₂ and C=C moieties, and as the Raman tweezers measured only molecules that remained on the optically trapped liposomes rather than being dissolved in solution, the decreased Raman intensities strongly indicate the cleavage of the acyl chain of lipids caused by the oxidative attack of hydroxyl radical.
Molecular oxygen (O₂), yielding a peroxyl radical. The resulting allylic radical can then couple with an allylic hydrogen from the unsaturated lipid by hydroxyl abstraction. As illustrated in Figure 5C, the reaction might begin with the abstraction of the hydroxyl radical, which can produce varied radicals that complicated chain reactions. The observation of numerous lines in mass spectra indicates that complicated reactions were involved and diverse products arose from the treatment of lipids with hydroxyl radicals. This result is expected because hydroxyl radical is highly reactive and can produce varied radicals that complicated chain reactions. We propose further a mechanism to account qualitatively for the cleavage of lipids caused by the attack of hydroxyl radical and the generation of fragments with decreased chain lengths.

To consolidate these deductions, we compared the mass spectra of lipids with and without treatment with hydroxyl radical. The mass spectrum of the control (without treatment) possessed only a single line corresponding to the molecular ion of the DOPC lipid (ratio of mass to charge, \( m/z = 785.93 \) u, Figure 5A), whereas that of the treated sample exhibited numerous lines corresponding to ions with masses smaller than that of the parent ion (Figure 5B). These results provide compelling evidence to confirm the deduction from the Raman measurements; that is, the treatment of DOPC with hydroxyl radical caused cleavage of the acyl chain of lipids.

Figure 5. Mass spectra and a proposed mechanism to account for the cleavage of lipids caused by treatment with hydroxyl radical. (A) Mass spectrum of lipids without treatment. (B) Mass spectrum of lipids measured after treatment of hydroxyl radical. (C) A proposed mechanism illustrating the cleavage of lipids caused by the attack of hydroxyl radical and the generation of fragments with decreased chain lengths.

Vitamins C and E Inhibit Alteration of Membrane Fluidity Induced by Lipid Peroxidation. We applied the same approach to elucidate the underlying inhibitory effects of vitamins C and E against the altered membrane fluidity induced by oxidative attack. We first measured the diffusion coefficients of DiD molecules on DOPC membranes treated with vitamin C only (1 mM, the control), or cotreated with vitamin C (1 mM) and hydroxyl radical (0.05 mM). The results, displayed in Figure 6A, show that the two diffusion coefficients varied insignificantly (\( D = 5.80 \times 10^{-8} \) cm² s⁻¹ vs 6.08 \( \times 10^{-8} \) cm² s⁻¹, \( P = 0.494 \)) in contrast to the result shown in Figure 3C for which the treatment with hydroxyl radical caused a significant increase in membrane fluidity. Co-treatment of vitamin C has effectively inhibited the change of membrane fluidity induced with hydroxyl radical.

Following the same approach, we examined the Raman spectra of single liposomes. As shown in Figure 6B, the temporally variation of Raman intensity at 1650 cm⁻¹ (C=C stretching mode) remains virtually constant (\( P = 0.43962 \)). This invariant Raman line shows unambiguously that vitamin C effectively prevented the oxidation of unsaturated lipids. This observation is consistent with the corresponding result of membrane fluidity (Figure 6A) thereby confirming that antioxidation is the underlying mechanism for vitamin C to inhibit
Model Lipid Membranes under Oxidative Attack

Figure 6. Effect of antioxidants on the fluidity and Raman spectra of vesicles under oxidative attack. (A) Comparison of diffusion coefficients of DiD molecules on DOPC membranes treated with vitamin C only (empty bar), or cotreated with vitamin C and hydroxyl radical (solid black bar). (B) Temporal evolution of the Raman line at 1650 cm\(^{-1}\) (gray, liposome treated with vitamin C only \(n = 5\); black, liposome cotreated with vitamin C and hydroxyl radical \(n = 4\)). (C) Comparison of diffusion coefficients of DiD molecules on DOPC membranes incorporating vitamin E before (empty bar) and after (solid black bar) treatment with hydroxyl radical. (D) Temporal evolution of the Raman line at 1650 cm\(^{-1}\) measured on a liposome incorporating vitamin E (gray, control \(n = 9\); black, treated with hydroxyl radical \(n = 4\)). **P < 0.01, *P < 0.05, NS P > 0.05, insignificant.

The increased membrane fluidity induced by hydroxyl radical. Although this conclusion is conventional wisdom,\(^{47,48}\) this result demonstrates that correlated Raman and FCS measurements provide a structural account of the modification of membrane fluidity under oxidative attack.

The result of corresponding measurements on DOPC membranes incorporating vitamin E is displayed in Figure 6C, which shows that vitamin E exhibited a similar capability in maintaining the fluidity of membranes under attack from hydroxyl radical (\(D = 6.00 \times 10^{-8}\) vs \(5.76 \times 10^{-8}\) cm\(^2\) s\(^{-1}\), \(P = 0.081\)). We then employed Raman spectral measurements to determine whether antioxidation or membrane stabilization is responsible for the unchanged membrane fluidity. According to the data shown in Figure 6D, the intensity of the Raman line at 1650 cm\(^{-1}\) of liposomes incorporating vitamin E remained unaltered in the presence of hydroxyl radical. This result clearly shows that vitamin E bound to the membrane effectively inhibited the oxidation of lipids and accounts satisfactorily for the observed intact membrane fluidity.\(^{48}\) Despite our result not excluding a contribution from increased stability due to the incorporation of vitamin E, the result indicates that this mechanism might not be essential to maintain fluidity for membranes under the attack of oxidative reagents.

Effect of Cholesterol on the Altered Membrane Fluidity Induced by Oxidative Attack. We measured the fluidity of membranes comprising a binary mixture of lipid and cholesterol with varied ratios. Figure 7 shows the diffusion coefficients of DiD molecules plotted as a function of the molar ratio of cholesterol in total lipids, whereas the inset shows the autocorrelation curves used to derive these diffusion coefficients. For clarity, only four curves (molar ratios of cholesterol in total lipids 0, 10, 25, and 50%) are displayed. The membrane fluidity clearly exhibits a gradual decrease with increasing content of cholesterol, except that the trend seems to become saturated in the range between 20 and 25%. The behavior resembling a phase transition indicates an onset of the formation of cholesterol-rich domains on membranes. A similar trend has been observed for membranes comprising cholesterol and various lipids; our observation agrees both qualitatively and quantitatively with the reported result.\(^{19}\)

We examined whether the cholesterol ratio in lipid membranes affects the change of membrane fluidity due to oxidative attack. A comparison of the diffusion coefficients measured before (white) and after (black) treatment with hydroxyl radical for membranes comprising varied cholesterol ratios (molar ratios of cholesterol in total lipids 0, 10, 25, and 50%) is shown in Figure 8A. The increase in membrane fluidity that was caused by oxidative attack showed a strong dependence on the ratio of cholesterol in lipid membranes. The fluidity of membranes that comprised little cholesterol (such as 0 and 10%) exhibited a great increase after exposure (60 min) to hydroxyl radical, whereas the fluidity increased to a smaller extent under the same treatment for membranes containing more cholesterol (62% increase for a membrane with 0% of cholesterol, \(P = 5.87 \times 10^{-8}\); 34% increase for a membrane with 10% of cholesterol, \(P = 2.91 \times 10^{-7}\); 14% increase for a membrane with 25% of cholesterol, \(P = 0.0281\)). Most significantly, the treatment with hydroxyl radical showed no discernible effect on the fluidity when the cholesterol ratio of membranes was increased to 50% (3.88 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1} \text{ vs} 3.99 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}, P = 0.461609). The incorporation of cholesterol into lipid membranes partially inhibited (10 and 25% of cholesterol), or entirely prevent (50% of cholesterol), an increased fluidity of a membrane caused by the oxidative attack from hydroxyl radical.

This novel inhibitory effect of cholesterol to the increased membrane fluidity caused specifically by the oxidative attack has never been reported. To elucidate the underlying mechanism and to demonstrate further the capability of the combined FCS and Raman approaches, we measured Raman spectra of liposomes comprising cholesterol of varied content under treatment with hydroxyl radical and the result is shown in Figure 8B. Similar to the result obtained on liposomes made of pure DOPC, the temporally variation of Raman intensity at 1650 cm\(^{-1}\) (\(\nu = \text{C=C stretching mode}\) exhibited a gradual decrease for liposomes comprising both DOPC and cholesterol. Comparison of the results displayed in Figure 8B further shows that the extent of
membrane fluidity decreased greatly when the fraction of cholesterol exceeded 30%, and as we have related the decreased fluidity to a phase transition, the above finding can be attributed at least in part to the onset of a phase transition that might accompany the formation of microdomains rich in cholesterol.49 We conclude that in addition to the capacity of antioxidation the formation of microdomains or a phase transition occurring in membranes with much cholesterol might also play roles in preventing membranes from a greatly increased fluidity under the attack from hydroxyl radical.

This strong dependence of the alteration of membrane fluidity induced by oxidation on the cholesterol content indicates that the cholesterol content of biological membranes (in cells and intracellular organelles) might have a pronounced effect on the ultimate fate of membranes under attack from oxidizing agents, as far as membrane fluidity is concerned. According to our results, membranes with small cholesterol content tend to have a significantly increased fluidity under oxidative attack. The percentage of cholesterol in the total lipids of mitochondrial membranes is only 10%,7 and the mitochondrion is particularly prone to oxidative attack due to its proximity to the site of ROS production.50.51 Our results suggest that the mitochondrion is not only vulnerable to oxidative attack but also susceptible to greatly altered membrane fluidity. As many vital mitochondrial activities are facilitated by enzymes bound to its membrane, drastic alteration of membrane fluidity would presumably compromise these functions. In contrast, lipid membranes comprising more cholesterol (such as myelin sheath27) might possess a resistance to the change of membrane fluidity, and survive under oxidative attack.

Conclusions

Using FCS and Raman measurements, we have characterized the lateral fluidity of membranes and the structure of lipids in model membranes under oxidative attack. Hydroxyl radical caused a significantly increased membrane fluidity. We demonstrated the ability of vitamins C and E to inhibit this ROS-induced alteration of membrane fluidity. All these results are explained according to the structural modification of lipids deduced from Raman spectra. We identified a novel resistance of membranes with much cholesterol against ROS-induced alteration of membrane fluidity and discussed its implication to cellular oxidative injury. Our results demonstrate the unique capability of the parallel approach based on FCS and Raman measurements to reveal an interplay between the structure and fluidity of membranes.

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References and Notes

Model Lipid Membranes under Oxidative Attack


