Effects of Lutein and Cholesterol on Alkyl Chain Bending in Lipid Bilayers: A Pulse Electron Spin Resonance Spin Labeling Study

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ABSTRACT A short pulse saturation recovery electron spin resonance technique has been used to study the effects of polar carotenoid-lutein and cholesterol on interactions of ^{14}N:^{15}N stearic acid spin-label pairs in fluid-phase phosphatidylcholine (PC) membranes. Bimolecular collisions for pairs consisting of various combinations of [^{14}N]-16-, [^{14}N]-10-, [^{14}N]-7-, or [^{14}N]-5-doxylstearate and [^{15}N]-16-doxylstearate in dimyristoyl-PC (DMPC) or egg yolk PC (EYPC) membranes were measured at 27°C. In the absence and presence of lutein or cholesterol for both lipid systems, the collision rates were ordered as 16:5 < 16:7 < 16:10 < 16:16. For all spin-label pairs studied, interaction frequencies were greater in DMPC than in EYPC. Polar carotenoid-lutein reduces the collision frequency for all spin-label pairs, whereas cholesterol reduces the collision frequency for 16:5 and 16:7 pairs and increases the collision frequency in the membrane center for 16:10 and 16:16 pairs. The presence of unsaturated alkyl chains greatly reduces the effect of lutein but magnifies the effect of cholesterol in the membrane center. The observed differences in the effects of these modifiers on alkyl chain bending result from differences in the structure of cholesterol and polar carotenoid and from their different localization within the lipid bilayer membrane. These studies further confirm the occurrence of vertical fluctuations of alkyl chain ends toward the bilayer surface.

INTRODUCTION

Carotenoids are widespread in living organisms. They are synthesized by *Procaryota* and plants, but also occur in animals, including vertebrates (Bramley and Mackenzie, 1988). In the photosynthetic process, carotenoids play two well-established functions: light harvesting to increase the efficiency of the antenna system (Siefermann-Harms, 1985; Gillbro et al., 1988; Koyama, 1991) and preventing photodynamic destruction (Griffiths et al., 1955; Claes, 1954; Conn et al., 1991). Recently, a third function of the carotenoids as a factor stabilizing the native structure of pigment-protein complexes has been emphasized (Plumley and Schmidt, 1987; Kühlbrandt et al., 1994; McDermott et al., 1995; Cohen et al., 1995). The group of photosynthetic xanthophylls participating in the xanthophyll cycle are loosely bound to the functional proteins (Demmig-Adams and Adams, 1992). One of the components of that cycle, zeaxanthin, is present transiently within the lipid bilayer phase of the thylakoid membrane, where it regulates membrane fluidity (Gruszecki and Strzalka, 1991).

Animals, including humans, obtain carotenoids from foods. Nonpolar (e.g., β-carotene and lycophene) and polar (e.g., zeaxanthin and lutein) carotenoids are found in blood plasma, adipose tissue, liver, and muscle (Bendich and Olson, 1989; Rojas-Hidalgo and Olmedilla, 1993). The role of carotenoids in the prevention of cancer and coronary heart disease, as well as health promotion, is a rapidly expanding field of research (Olson, 1989; Sies et al., 1992; Krinsky, 1993).

Large amounts of carotenoids were found in human macula (Bone et al., 1985; Handelman et al., 1988). Human macular pigment is a mixture of two polar carotenoids, zeaxanthin and lutein (Bone et al., 1985); however, the last measurements show that the zeaxanthin fraction is a mixture of two stereoizomers, zeaxanthin itself and *meso*-zeaxanthin (Bone et al., 1993). Zeaxanthin and lutein are located in the macula in an environment that is very similar to that of liposomes (Bone et al., 1992), with their long axis perpendicular to the membrane surface (Bone and Landrum, 1984).

Recently, the role of carotenoids in the macula has been extensively investigated. Findings from a nutrition study support the possibility that increased dietary intake of carotenoids may reduce the risk of age-related macular degeneration (AMD), the leading cause of irreversible blindness among persons over 65 (Seddon et al., 1994; Seddon and Hennekens, 1994). The retina is prone to oxidative damage due to the high level of polyunsaturated fatty acids in the photoreceptor outer-segment membranes (Young, 1988). Light and normal metabolic processes within cells can generate free radicals and highly reactive species of oxygen such as singlet oxygen. The antioxidant activity of carotenoids as well as their ability to quench singlet oxygen are well established (Burton and Ingold, 1984; Krinsky, 1989; Foote et al., 1970); carotenoids could also serve such functions within the retina. On the other hand, the association between carotenoid intake and AMD may not be related to only an antioxidant mechanism, given that foods containing vitamins E and C, which also have antioxidant potential,

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were not significantly associated with AMD (Seddon et al., 1994).

In the present work, we extended our electron spin resonance (ESR) study of the effects of polar carotenoids on the membrane structure and dynamics to their effect on alkyl chain bending in saturated and unsaturated phosphatidylcholine (PC) membranes. Polar carotenoids are present in the lipid bilayer portion of thylakoid membranes and photoreceptor outer segment membranes and are selectively accumulated in the retina from the blood. Nonpolar carotenoids, such as β-carotene and lycophene, are absent in the retina (Schalch, 1992; Handelman et al., 1992). Additionally, we compared the effects of polar carotenoids with the effect of cholesterol, because both are thought to regulate membrane fluidity, the former in *Procaryota* and the latter in *Eucaryota* (Rohmer et al., 1979).

Vertical fluctuations of phospholipid alkyl chains were investigated by different techniques: continuous wave electron-electron double resonance (CW ELDOR) (Feix et al., 1984), fluorescence quenching (Merkle et al., 1987; Wardlaw et al., 1987), and saturation recovery ESR (Yin et al., 1987b). We used short-pulse saturation-recovery ESR to study the collisions between ^{14}N:^{15}N stearic acid spin-label (SASL) pairs (Yin et al., 1987a). The ^{15}N nitroxide moiety was always attached at the C16 position of the stearic acid molecule, whereas the position of the ^{14}N nitroxide moiety was at C16, C10, C7, or C5. The interaction between the $^{14}NC16$:$^{15}NC16$ pair is dependent primarily on the lateral diffusion of stearic acid spin labels, whereas the interaction between $^{14}NC10$:$^{15}NC16$, $^{14}NC7$:$^{15}NC16$, and $^{14}NC5$:$^{15}NC16$ pairs requires a vertical fluctuation of the nitroxide moiety that is at the C16 position toward the polar surface of the membrane. Fig. 1 illustrates the chemical structures of lutein, cholesterol, and spin labels and their approximate location in the lipid bilayer composed of saturated dimyristoyl- or unsaturated egg yolk phosphatidylcholines (DMPC or EYPC, respectively). The possible collisions between nitroxide moieties are also indicated.

MATERIALS AND METHODS

The 16-doxylstearic acid spin label (16-SASL) containing the ^{15}N-substituted nitroxide moiety at the 16 position ($^{15}NC16$) was synthesized according to the method of Joseph and Lai (1987). The corresponding ^{14}N

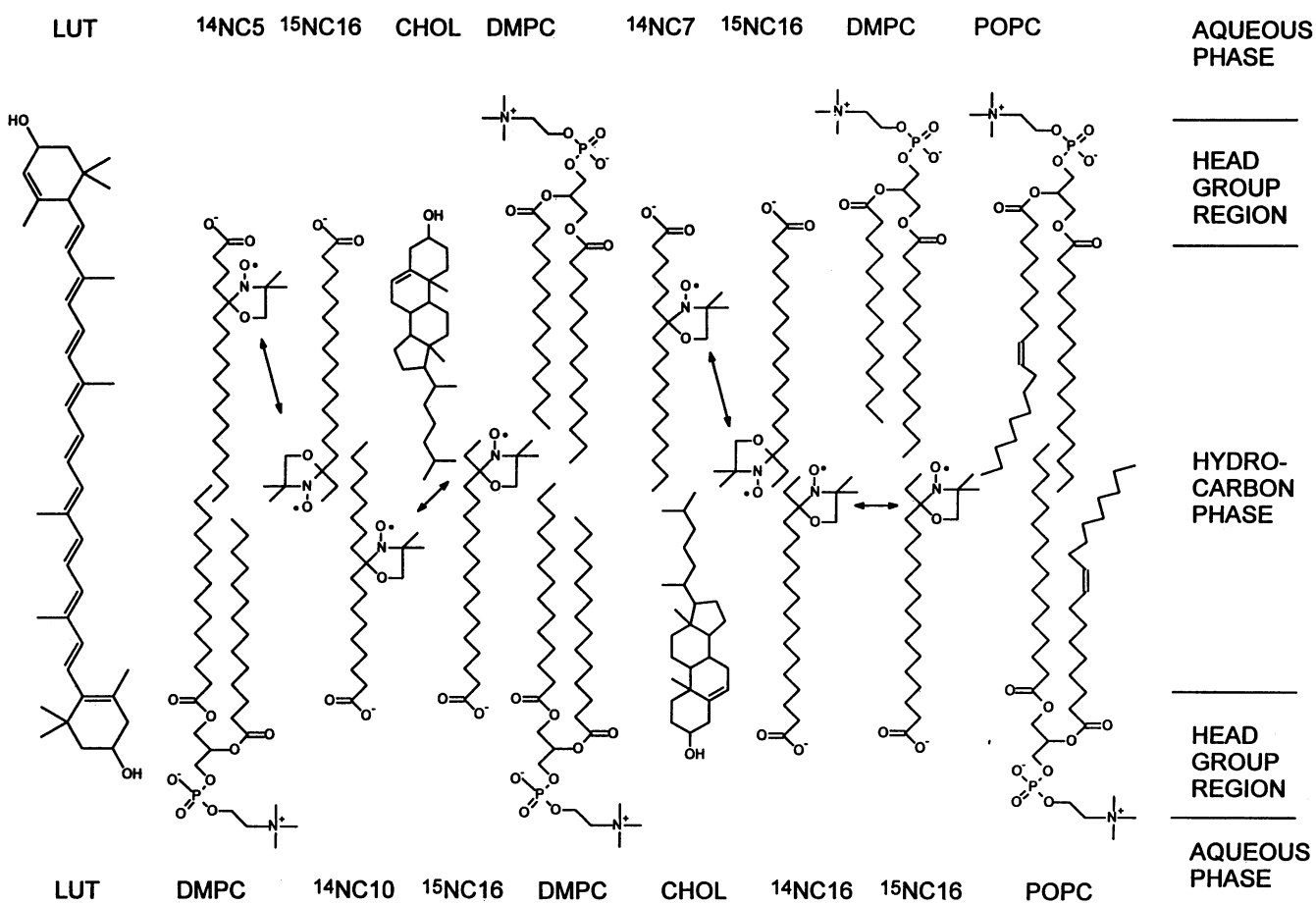

FIGURE 1 Cross-sectional drawing of the lipid bilayer including lutein, cholesterol, and spin labels. Observed collision between ^{14}N:^{15}N spin-label pairs are indicated. DMPC and 1-palmidoyl-2-oleoyl PC (POPC) molecules are also shown. POPC represents the major component (70%) of the EYPC mixture (Seelig and Waespa-Šarčevič, 1978).

n-SASLs (^{14}NC5, ^{14}NC7, ^{14}NC10, and ^{14}NC16) were obtained from Molecular Probes (Eugene, OR). DMPC and EYPC were purchased from Sigma Chemical Co. (St. Louis, MO), cholesterol (crystallized) was from Boehringer-Mannheim Diagnostic (Indianapolis, IN), and lutein was from Kemin Industries (Des Moines, IA); these were used without further purification. The concentration of lutein was determined spectrophometerically, using the extinction coefficient given by Davies (1974).

The membranes used in this work were a multilamellar dispersion of investigated phosphatidylcholine (PC) containing 0.5 mol% SASL (^{14}N and/or ^{15}N) in the absence or presence of 10 mol% lutein or 20 mol% cholesterol. Membranes were prepared by the following method of Kusumi et al. (1986) and Subczynski et al. (1992). Chloroform solutions of the lipids, carotenoids, or cholesterol and spin labels were mixed (containing 0.5×10^{-5} mol of total lipids), and chloroform was evaporated with a stream of nitrogen gas and then further evaporated under reduced pressure (0.1 mm Hg) for at least 12 h. A buffer solution (0.1 ml) was added to the dried lipids at about 40°C and vortexed vigorously. The buffer (0.1 M borate, pH 9.5) was used to ensure that all SASL probe carboxyl groups were ionized in the PC membranes (Kusumi et al., 1986, and references therein). The lipid dispersion was centrifuged briefly at 12,000 × *g* for 15 min at 4°C, and the loose pellet (about 20% lipid, w/w) was used as a sample. All samples were run in capillaries made of the methylpentene polymer TPX (0.6 mm id). A flow of temperature-regulated nitrogen gas over the capillary was used to remove oxygen (Hyde and Subczynski, 1989). All preparations and measurements of samples with carotenoids were performed in the dark or in dim light.

Conventional ESR spectra were obtained with a Varian E-109 X-band spectrometer using Varian temperature control accessories. Spectra were obtained with 1 mW of incident microwave power and 100-kHz field modulation of 0.5 G. The saturation-recovery spectrometer used in this study is based on the design of Huisjen and Hyde (1974). The data-processing system was recently reconstructed using commercially available fast digital signal processors (model TRAQH system; DSP Technology). The timing of the spectrometer is controlled by a 200-MHz oscillator (model CO-431A-OX; Vectron) that is also used as the external clock of the transient recorder. Two 10-MHz outputs trigger the pulse generators (model DG 535; Stanford Research System). The averager supports an addition and subtraction mode for suppression of switching transients and baseline corrections using field modulation. A field-effect transistor microwave amplifier (model AMF-3S-8596-68; MITEQ) has been introduced. The time response limit is 0.1 μs. A high-order, low-pass filter at the input to the analog/digital converter cuts off at 25 MHz, which results in minimal distortion at the transient signal. The loop-gap resonator (1 mm diameter of the loop and 10 mm length) has been used. The saturating pulse power in the rotating frame was about 2 G. All data were obtained with 50-Hz pump biphase modulation for suppression of free-induction decay signal and 25 Hz field modulation for improving the quality of saturation recovery signal by reducing the instrumental artifacts. In most cases the measurements of T_1 were made on the low field line ($m_I = 1/2$) of the ESR spectra of the ^{15}N spin label, because of the higher sensitivity. For samples containing single ^{14}N species of SASL, the measurements were made on the central line ($m_I = 0$) of the ESR spectra. Typically, 5×10^3 decays per second were acquired, with 512 data points per decay. The total accumulation time was 2 min. Aperture intervals were 20 or 50 ns.

In general, where there are interacting ^{14}N and ^{15}N spin systems, the saturation-recovery experiment is expected to give five superimposed exponentials (Yin et al., 1987b). However, when $W_n \gg W_e$, the nuclear spin states within each isotopic system are strongly coupled and a simplified relaxation model can be used in which only two superimposed exponentials are observed (Yin et al., 1988). The validity of this assumption was checked using CW ELDOR (Yin et al., 1990).

The saturation recovery signal is (Yin et al., 1988)

$$i = I_1 e^{-(A-B)t} + I_2 e^{-(A+B)t}, \tag{1}$$

where

$$A = W_{12} + W_{34} + K_x(N_I + N_{II})/2, \tag{2}$$

and

$$B = [(W_{12} - W_{34})^2 - K_x(N_I - N_{II})(W_{12} - W_{34}) + (1/4)K_x^2(N_I - N_{II})^2]^{1/2}. \tag{3}$$

W_{12} and W_{34}, the spin-lattice relaxation rates ($2W_e = 1/T_{1e}$) of the ^{14}N and ^{15}N spin labels, were measured independently by saturation recovery in membranes containing either the ^{14}N or ^{15}N spins alone (Tables 1 and 2). N_I and N_{II} are the ^{14}N and ^{15}N spin label concentrations, respectively, expressed as mol% of the total phospholipid. Computer modeling of the experimental saturation recovery curve for a given ^{14}N:^{15}N pair gives a double exponential with two time constants from which the collision rate constant, K_x, can be calculated.

The theoretical models were compared with experimental saturation-recovery curves using the damped least-squares method, which has proved successful for fitting overlapping exponential decay curves (Laiken and Printz, 1970) and ESR spectra (Pasenkiewicz-Gierula et al., 1987). The curve-fitting program was run on an IBM PC/486 computer.

The short-pulse saturation-recovery ESR experiments were performed as in our previous studies (Yin et al., 1987a, 1990; Subczynski et al., 1989). As a control, every experiment was run with different pump durations because the time constants at the multiexponentials are independent of the width of the pump pulse, which only changes the prefactor of the multiexponentials (Yin et al., 1987a,b). When the pulse width for the same sample is varied, the computer fitting provides the same time constants but different prefactors. This approach has been used to obtain higher quality of the fitting and more reliable data.

RESULTS

Conventional ESR spectra for the various combinations of ^{14}N-SASLs and ^{15}NC16 in EYPC membranes at 27°C are shown in Fig. 2. For samples in which the motion of the ^{14}N probe is relatively fast, the ^{14}N and ^{15}N resonances in the low field region are reasonably well resolved. For the pairs, where there is partial spectral overlapping throughout the spectra, the short pulse saturation recovery method will give reliable bimolecular collision rates (Yin et al., 1988). To a good approximation (for homogeneous line, $T_1 \gg T_2$), saturation-recovery ESR depends only on the time evolution of the *z*-component of magnetization, the eigenvalues of the matrix, i.e., the time constants of the decay signal will be independent of spectral overlap if the multiple exponentials can be separated. As indicated in our earlier papers, the high

TABLE 1 Electron spin lattice relation time (T_{1e}) for ^{14}N or ^{15}N SASL in DMPC bilayer at 27°C (μs)

Additions	^{14}NC5	^{14}NC7	^{14}NC10	^{14}NC16	^{15}NC16
None	4.92 ± 0.10	4.43 ± 0.08	4.38 ± 0.07	2.39 ± 0.07	2.78 ± 0.09
10 mol% lutein	5.38 ± 0.07	5.43 ± 0.08	4.83 ± 0.09	2.58 ± 0.12	3.03 ± 0.04
20 mol% cholesterol	5.43 ± 0.05	4.99 ± 0.11	3.61 ± 0.10	2.11 ± 0.05	2.37 ± 0.09

TABLE 2 Electron spin lattice relation times (T_{1e}) for ^{14}N or ^{15}N SASL in EYPC bilayer at 27°C (μs)

Additions	^{14}NC5	^{14}NC7	^{14}NC10	^{14}NC16	^{15}NC16
None	4.97 ± 0.10	4.91 ± 0.08	4.80 ± 0.09	2.47 ± 0.05	2.84 ± 0.12
10 mol% lutein	5.07 ± 0.06	5.35 ± 0.10	4.95 ± 0.05	2.65 ± 0.11	2.93 ± 0.03
20 mol% cholesterol	5.08 ± 0.04	5.28 ± 0.14	4.61 ± 0.07	2.17 ± 0.06	2.63 ± 0.05

Samples contained a single species of either ^{14}N or ^{15}N SASL present in the liposomes at 0.5 mol%. The buffer was 0.1 M borate, pH 9.5. Saturation-recovery experiments were performed using a relatively long (5 μs) saturating pulse, after which single exponentials were obtained. Time constants were obtained from computer simulation.

quality of the saturation recovery ESR data and the numerous experimental controls (such as by varying the pulse width) that are available demonstrate that this will often be possible.

Spin-lattice relaxation times for the individual species of SASLs in EYPC and DMPC membranes are given in Tables 1 and 2. For pure membranes, T_{1e} values become larger as effective rotational correlation times increase (C5 ≥ C7 > C10 > C16), as expected from either spin-rotational (Atkins and Kivelson, 1966) or electron-nuclear dipolar (Bloembergen et al., 1943; McConnell, 1956) mechanisms. We also note that the T_{1e} for a given ^{14}N- or ^{15}N-SASL is always slightly greater in unsaturated (EYPC) versus saturated (DMPC) membranes. The presence of the rigid *cis* double bond in the alkyl chain decreases the lateral diffusion constant of lipids but not the rotational correlation time of spin labels (Kusumi et al., 1986). These results are in agreement with the latest theoretical development on spin-lattice relaxation of nitroxide spin labels (Robinson et al., 1994), in that the isotropic Brownian translational motion is also the motional mechanism involved in the spin lattice relaxation process. We confirm the previous result that T_{1e} for a given ^{15}N-SASL is always greater than that of the corresponding ^{14}N-SASL (Yin et al., 1990).

The addition of lutein increases T_{1e} for all SASLs in both saturated and unsaturated membranes; however, the effect of cholesterol is complex. It increases T_{1e} for 5- and 7-SASL and decreases T_{1e} for 10- and 16-SASL. Conventional ESR spectra always show decreased motion in the presence of cholesterol (Kusumi et al., 1986). The opposite effect of lutein and cholesterol on T_{1e} for 16-SASL is clearly seen in Fig. 3. Cholesterol increases the relaxation rate, whereas lutein decreases it. This effect is more pronounced in DMPC than in EYPC.

A representative saturation-recovery signal, along with a simulated fit, is shown in Fig. 4 for a ^{14}NC10:^{15}NC16 spin-label pair in DMPC at 27°C. The curve was fit to a single exponential (Fig. 4 *A*) and to a double exponential

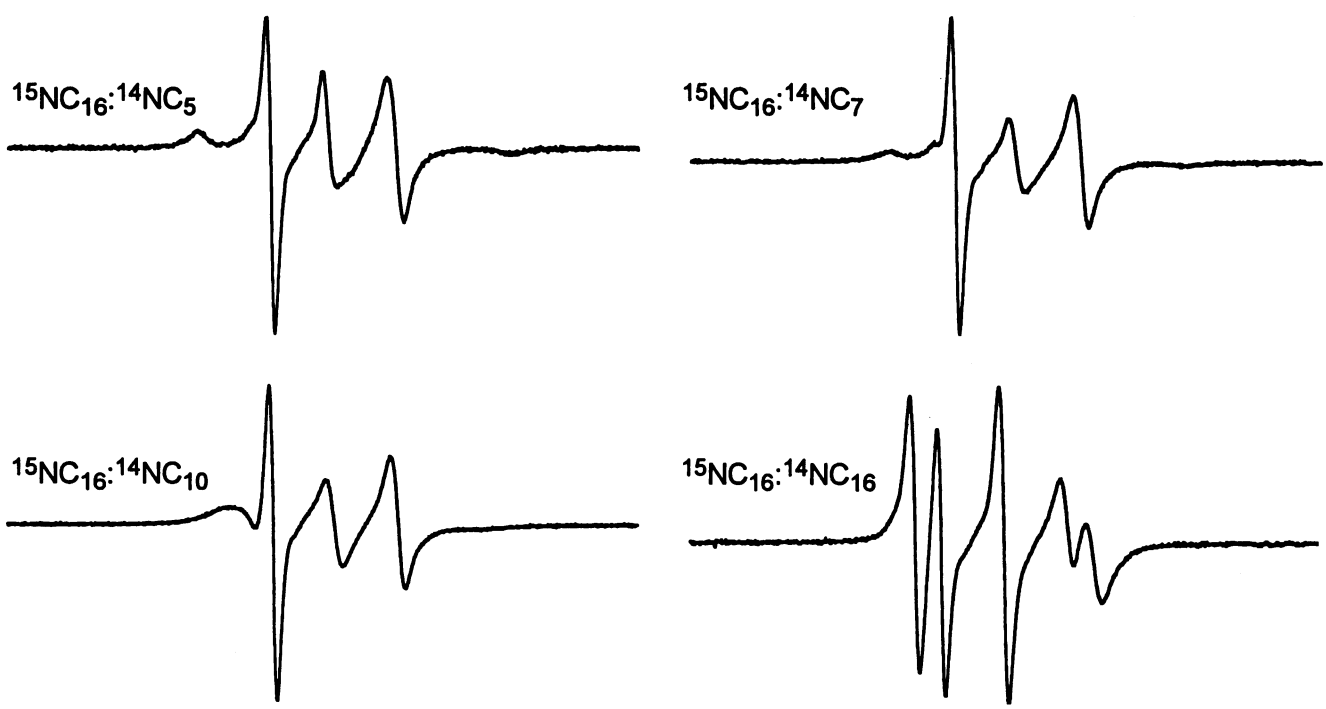

FIGURE 2 ESR spectra of four combinations of ^{15}N and ^{14}N *n*-SASL. Samples contain 0.5 mol% ^{14}N spin label and 0.25 mol% ^{15}N spin label in EYPC equilibrated with 0.1 M sodium borate, pH 9.5, at 27°C. The lower concentration of ^{15}N spin label was used to show the better ratio of the line intensities in the figure.

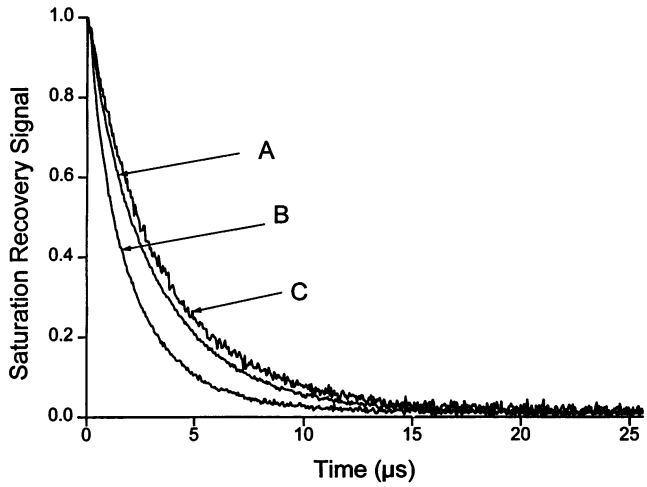

FIGURE 3 Typical saturation-recovery signal of ^{14}NC16 in DMPC membranes at 27°C. T_{1e} = 2.39, 2.11, and 2.58 μs in the absence of modifiers (*A*) or the presence of 20 mol% cholesterol (*B*) or 10 mol% lutein (*C*), respectively.

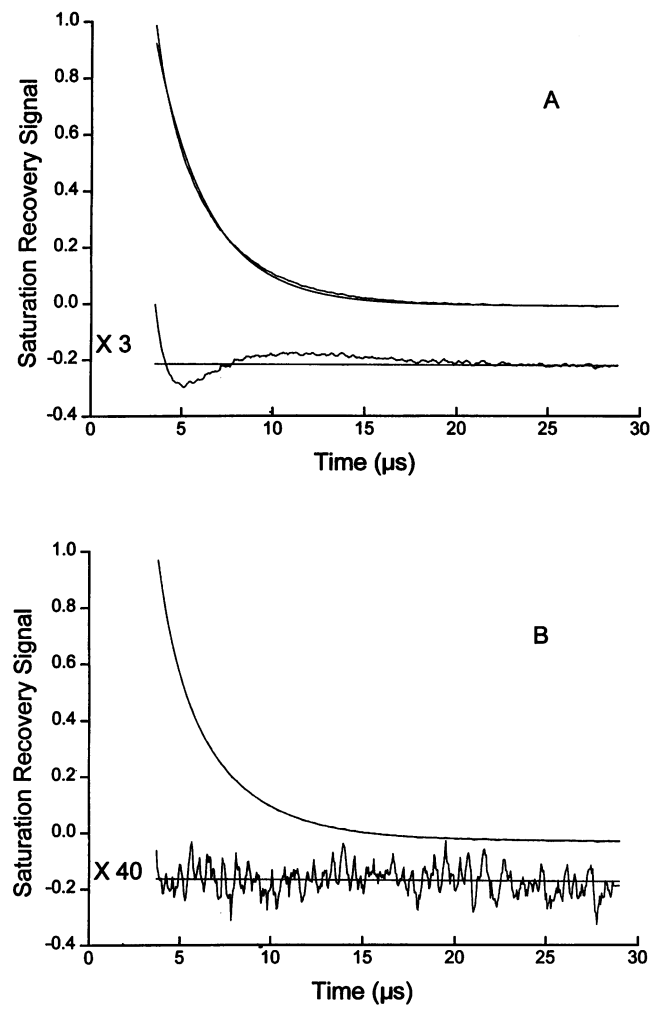

FIGURE 4 Saturation-recovery signal and curve fitting for ^{14}NC10: ^{15}NC16 (0.5:0.5 mol%) in DMPC at 27°C, pH 9.5. The recovery curve contains 512 data points with a resolution of 50 ns per point. Simulations and experimental saturation-recovery signals are superimposed. The differences, multiplied by a factor of 3 for single-exponential fitting (*A*) and by a factor of 40 for double-exponential fitting (*B*), are shown as the "residual" beneath the recovery curve. Each recovery signal was obtained in 2 min at 6000 accumulations/s. The time constants are 3.38 μs and 0.97 μs from the double exponential fitting.

(Fig. 4 *B*). The Heisenberg spin exchange-rate constant, K_x, was calculated from the two recovery time constants obtained from the fit to a double exponential and the known spin-lattice relaxation rates (Tables 1 and 2), using Eqs. 2 and 3. All experiments were repeated three times with the pulse width 0.2, 0.3, and 0.5 μs for each sample. The data were obtained with an error of less than 5%. Fig. 4 *B* shows the typical saturation-recovery signal and the accuracy of the curve fitting.

The bimolecular collision frequencies between ^{15}N-C16 and the various positioned isomers of the ^{14}N-SASL are shown in Fig. 5 for DMPC and in Fig. 6 for EYPC membranes. In pure membranes, the interaction frequency was directly dependent on the relative separation between the nitroxide moieties along the alkyl chain, decreasing with increasing separation of the nitroxide groups. Collision frequencies are always greater in DMPC than in EYPC membranes. This result is in agreement with our previous observation using CW ELDOR (Feix et al., 1987).

The addition of 10 mol% lutein decreases the bimolecular collision frequencies between 16-SASL and other spin labels. For all collisions, the effect of lutein is greater in saturated than in unsaturated membranes. The addition of 20 mol% cholesterol increases the bimolecular collision frequency between ^{15}NC16-^{14}NC16 and ^{15}NC16-^{14}NC10 and decreases it between ^{15}NC16-^{14}NC7 and ^{15}NC16-^{14}NC5. Cholesterol facilitates collisions in the membrane center, whereas collision rates decrease close to the membrane surface. The presence of unsaturated alkyl chains greatly changes the effects of both modifiers in the membrane center. In the DMPC bilayer, lutein decreases the collision frequency between ^{15}NC16 and ^{14}NC16 by 41% and between ^{15}NC16 and ^{14}NC10 by 33%. In the EYPC bilayer, these collision frequencies decrease by only 18% and 21%, respectively. Cholesterol in the DMPC bilayer, however, increases the collision frequency between ^{15}NC16 and ^{14}NC16 by only 10% and between ^{15}NC16 and ^{14}NC10 by only 18%. In the EYPC bilayer, cholesterol increases these collision frequencies by 26% and 25%, respectively.

DISCUSSION

In our earlier papers (Subczynski et al., 1992, 1993) we showed that the effects of polar carotenoids on the structure and dynamic properties of lipid bilayer membranes are, in many aspects, similar to the effects of cholesterol. Both increase the order and decrease the alkyl chain motion (observed with a conventional ESR spin-labeling method) in fluid-phase membranes and disorder lipids in gel-phase membranes. Both are known to broaden the gel-to-fluid

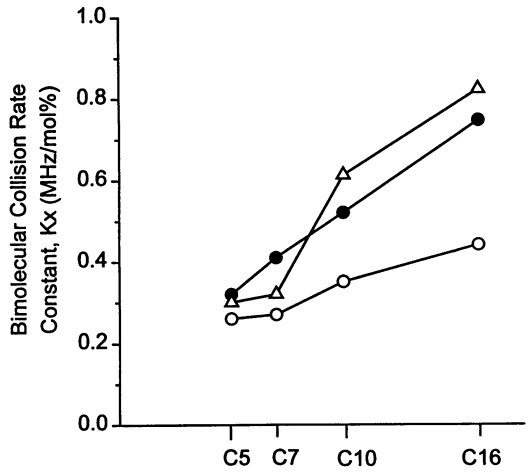

FIGURE 5 Bimolecular collision rate constants for a nitroxide moiety at the C16 position of the stearic acid alkyl chain with other SASLs in (●) DMPC alone, (△) DMPC with 20 mol% cholesterol, and (○) DMPC with 10 mol% lutein at 27°C.

phase transition and increase the mobility of polar headgroups. A quantity of 10 mol% polar carotenoids exerts an effect similar to that of 15–20 mol% cholesterol. As a rule, the presence of unsaturated alkyl chains moderates the effect of polar carotenoids and cholesterol (Lippert et al., 1980; Kusumi et al., 1986; Subczynski et al., 1992, 1993). The ordering effect of cholesterol does not depend on the bilayer thickness (Kusumi et al., 1986). In contrast, the relationship of the length of the carotenoid molecule to the thickness of the PC membrane is a significant factor, determining the effect of polar carotenoids on membrane fluidity (Subczynski et al., 1993).

The results presented in this paper, as well as those in earlier works dealing with measurements of the oxygen transport within the lipid bilayer (Subczynski et al., 1989, 1991a,b), show additional significant differences between the effects of both modifiers on membrane dynamics. We summarize the results of both types of measurements as follows:

1. The presence of unsaturated alkyl chains decreases the frequency of alkyl chain bending and reduces the oxygen transport at all locations in the membrane.

2. Intercalation of polar carotenoids in PC membranes decreases the frequency of alkyl chain bending (the effect in an unsaturated PC membrane is weaker than in a saturated one) and reduces the oxygen transport at all locations in the membrane.

3. Intercalation of cholesterol in PC membranes decreases the frequency of vertical fluctuations of the end of alkyl chains near the polar headgroup region, but increases the frequency of chain bending in the membrane center. In parallel, it decreases oxygen transport in and near the polar headgroup regions and increases the oxygen transport in the membrane center. The presence of unsaturated alkyl chains greatly magnifies these effects of cholesterol in the membrane center.

These differences result from the different structures and the different locations of cholesterol and polar carotenoids in the membrane (see Fig. 1). The molecule of cholesterol is located in one leaflet of the bilayer, and its rigid plate-like portion extends to a depth of carbon atoms 7–10 in lipid alkyl chains (McIntosh, 1978). The cross section of the isooctyl chain of the cholesterol molecule is much smaller than the cross section of the rigid steroid ring and, therefore, produces additional possibilities for undulation and *trans-gauche* transitions of alkyl chains in the membrane center. In contrast, one polar carotenoid molecule influences both halves of the lipid bilayer, because two polar groups interact with opposite hydrophilic surfaces of the membrane. Both vertical fluctuations of the ends of the alkyl chains and oxygen transport are reduced in those regions of the lipid bilayer membranes to which the rigid portion of molecule of the modifier (cholesterol or polar carotenoid) extends.

The present results are in agreement with out earlier observations (Subczynski et al., 1989, 1991a) that cholesterol increases the fluidity in the membrane center. This region is additionally characterized by the very high hydrophobicity (Subczynski et al., 1994). This suggests the possibility of lateral transport of small nonpolar molecules along the inner core region of the membrane (parallel to the membrane surface). It can be speculated that the mismatch between the length of cholesterol (the bulky fused-ring structure of cholesterol, in particular) and the PC molecule would tend to create more free volume for *trans-gauche* transitions in the membrane center, which would increase oxygen transport in this region. Our speculation is supported by the observations of Bush et al. (1980) and Pink et al. (1981) that in the presence of cholesterol the average number of *gauche* bonds in the hydrocarbon chain of dipalmitoyl-PC changes slightly, if at all. Their results suggested an increase in the number of *gauche* bonds in the center of the membrane, because the part of the alkyl chain

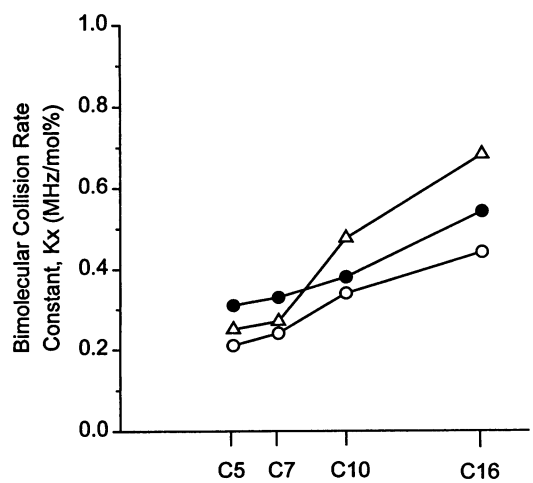

FIGURE 6 Bimolecular collision rate constants for a nitroxide moiety at the C16 position of the stearic acid alkyl chain with other SASLs in (●) EYPC alone, (△) EYPC with 20 mol% cholesterol, and (○) EYPC with 10 mol% lutein at 27°C.

encountering the steroid nucleus shows an increase in the motional order that corresponds to a decrease in the *trans-gauche* transition (Stockton and Smith, 1976). Polar carotenoids reduce the *trans-gauche* isomerization by enhancing the *trans* conformation of the alkyl chain along its entire length, which reduces oxygen transport.

There is a seeming discrepancy between our observations that cholesterol increases the frequency of the alkyl chain bending and oxygen transport and decreases T_1 of 16-SASL near the center of the membrane and the well-established ordering effects of this molecule, which extend to the whole alkyl chain of the lipid bilayer (Stockton and Smith, 1976; Shin and Freed, 1989: Subczynski et al., 1991b). However, it is pertinent that the order parameter indicates a static property of the lipid bilayer, whereas collisions between free radical fragments of stearic acid spin labels, oxygen transport, and T_1 of spin labels characterize its dynamics. Moreover, deviations in the alkyl chain segment direction from the bilayer normal accumulate as one proceeds from the bilayer surface to the membrane interior, a result of the effective tethering of the alkyl chain at the bilayer surface. Consequently, ordering of the alkyl chain induced by the steric contact with the plate-like portion of cholesterol will also cause ordering of the distal fragment of the alkyl chain, even though the number of *gauche* bonds is not changed in that fragment. Therefore the increased mobility induced by cholesterol in the membrane center, indicated by the dynamic spectroscopic characteristics, is not inconsistent with increased order, a static membrane property.

In 1981 Barclay and Ingold emphasized the significance of the vertical fluctuation of lipid peroxyl radicals in the termination of the lipid peroxidation chain reactions by vitamin E. Other processes involved in lipid peroxidation could also occur at the membrane-water interface. They are initiation of lipid peroxidation, which requires hydrogen abstraction from unsaturated lipid carbon-carbon bonds, and secondary initiation from lipid hydroperoxides. Both are promoted by reactions with certain metal ions and water-soluble free radicals (Borg, 1995). Vertical fluctuation of lipid alkyl chains toward the membrane surface should thus be taken into account in the investigation of chemical reactions involved in lipid peroxidation.

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