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Effects of Fumonisin B_1 on Oxygen Transport in Membranes

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Electron spin resonance (ESR) spin-label oximetry has been used to study the effects of fumonisin B_1 (FB_1), a sphingoid-like mycotoxin, on oxygen transport in phosphatidylcholine (PC) bilayers. Moreover, the use of spin labels attached to different carbons of fatty acids makes it possible to do structural and oximetric determinations with the same test sample. Specifically, the incorporation of 10 mol% FB_1 increased the oxygen transport properties of both saturated and unsaturated membranes at 37°C by *ca.* 30% and decreased the ordering of the hydrocarbon chains near the surface of the membranes; concomitantly, oxygen transport near the center of bilayers was diminished slightly, and the relative oxygen diffusion–concentration product profile curves were markedly flattened.

Subtle changes in oxygen availability often have a profound impact on the physiological and homeostatic processes within living organisms. Ongoing conceptual and methodological advancements concerning measurements of oxygen concentration, diffusion, transport, and related issues, *e.g.*, oxygen-mediated reactions, underscore the fundamental importance of this burgeoning field to studies pertaining to health and disease (1,2). Electron spin resonance (ESR) oximetry methods are based on bimolecular collisions of O_2 with spin labels, which effectively shorten the spin-lattice relaxation time and broaden the line width of the ESR signal. The subject of ESR spin label oximetry has been reviewed recently (3,4). Possible methods for observing these collisions, classified as T_1-sensitive or T_2-sensitive, are outlined in ref. 4. By far the best T_1-sensitive method is saturation-recovery ESR (5-7). The continuous wave (CW) saturation technique is adequate and instrumentally easy to use in comparison with the saturation recovery method (8-10), especially when relative--rather than absolute--measurements are to be made on test samples (with identical spin labels). One T_2-sensitive method, the line width method, is the most widely used for ESR oximetry, both *in vivo* and *in vitro*, because it is instrumentally easy (11-15).

The fumonisins, which are produced by *Fusarium* spp., are a relatively newly identified family of mycotoxins. They possess a lipophilic backbone closely related to the sphingolipid sphinganine. In light of epidemiological investigations that associate *Fusarium moniliforme*, which produce the fumonisins, with human esophageal cancer (16-19) as well as several animal feeding studies that demonstrate a causative role for these mycotoxins in leukoencephalomalacia in horses, pulmonary edema in swine, and hepatic cancer in rats, extensive efforts are directed toward elucidating their mode(s) of action (20,21). Cellular membranes are postulated to be one principal target for the fumonisins *in vivo.* The finding that the fumonisins are potent inhibitors of the enzyme sphinganine N-acyl transferase, *i.e.*, ceramide synthase in the endoplasmic reticulum, clarified one likely aspect of their toxicological activity (22,23).

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Abbreviations: FB_1, fumonisin B_1; DMPC, L-α-dimyristoylphosphatidylcholine; EYPC, egg yolk phosphatidylcholine; ESR, electron spin resonance; n-SASL, n-doxylstearic acid spin label (n = 5, 7, 10, 12, or 16); T-PC, 1,2-dioleoyl-*sn*-glycero-3-phosphotempocholine.

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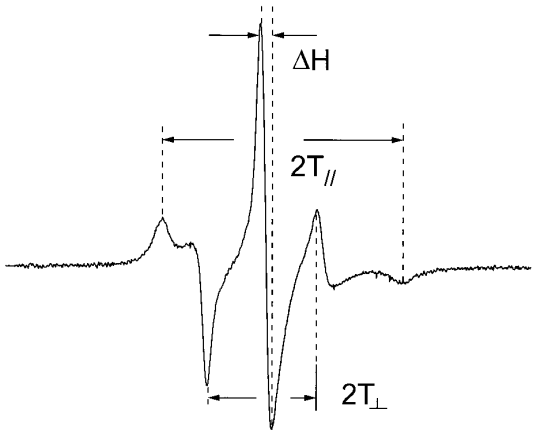

FIG. 1. Typical conventional ESR spectrum of 7-SASL in EYPC at 37°C, in 0.1 M Hepes buffer, pH 7.2.

Atherogenic effects of *Fusarium moniliforme* cultures in a non-human primate have been observed (24).despite a current appreciation of particular aspects of lipid metabolism with respect to the toxicological activity of the fumonisins, there do not appear to be any reports that specifically address their interactions with cellular membranes.

Because changes in oxygen concentration in living cells reflect the rate of oxygen consumption and degree of oxygen permeability of the plasma membrane, we have sought to determine whether FB_1 affects oxygen transport in membranes by using spin label CW-ESR spectroscopy (T_2-sensitive) methods to measure oxygen concentration products. In this work, we have carried out systematic studies on the influence of fumonisin B_1 on the structure and dynamic properties of phosphatidylcholine membranes. Multilamellar liposomes consisting of dimyristoylphosphatidylcholine (DMPC) or egg yolk phosphatidylcholine (EYPC) were used. Six different nitroxide spin labels were implemented to determine the effects of FB_1 on membrane structure and oxygen transport.

MATERIALS AND METHODS

Fumonisin B_1 (FB_1) was prepared as reported previously (25). It was isolated from cultures of *Fusarium proliferatum* strain M-5991 grown on corn and extracted with methanol/water (75/25). The extracted FB_1 was purified by using a series of preparatory liquid chromatography columns of C-18 and cyano phases (alternately). The water was removed from the purified FB_1 by freeze-drying. The ^{1}H-NMR data for purified FB_1 were consistent with those reported by Blackwell (26). The n-doxyl stearic acid spin labels (SASL)--containing ^{14}N-nitroxide moieties attached to carbons 5, 7, 10, 12, and 16 positions--and the lipids L-α-lecithin-(phosphatidylcholine) (EYPC) and L-α-dimyristoylphosphatidylcholine (DMPC) were purchased from Sigma Chemical Co. (St. Louis, MO). The spin label 1,2-dioleoyl-sn-glycero-3-phosphotempocholine (T-PC) was obtained from Avanti Polar Lipids, Inc. (Alabaster, AL).

The membranes used in this work were a multilamellar dispersion of investigated PC containing 0.5 mol% spin labels (n-SASL or T-PC) in the absence or presence of 10 mol% FB_1. Membranes were prepared according to methods of Feix *et al.* (27). All test samples were run in capillaries made of the methylpentene polymer TPX (0.7-mm i.d). This plastic capillary is permeable to oxygen and nitrogen and is substantially impermeable to water (28). The TPX capillary was placed inside the ESR dewar insert and equilibrated with a flow of various gases (nitrogen, oxygen, or air) that were also used for temperature control.

Conventional ESR spectra were obtained with a Varian E-109 X-band spectrometer and a variable temperature controller accessory. ESR signals were obtained with 2 mW incident microwave power and 100 KHz field modulation of 1G (for order parameter) and 0.1G (for line broadening) measurements. Spectra were recorded, stored, and manipulated in an IBM/PC computer. A total of 1024 data points were taken by using a scan width of 100 G (for order parameter) and 10 or 20 G (for line broadening).

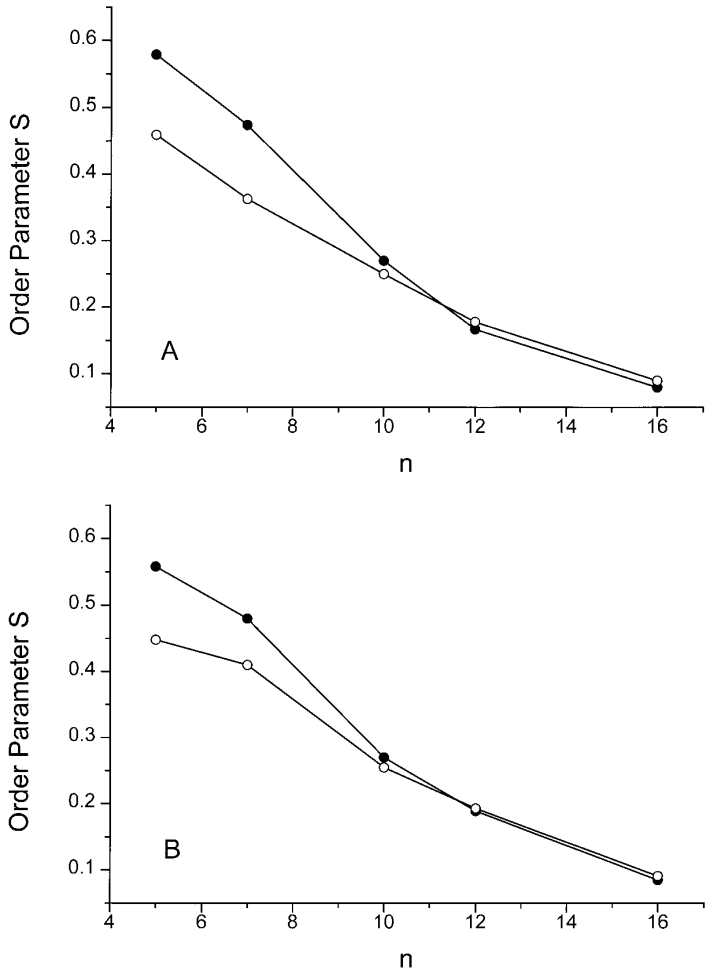

FIG. 2. Order parameters S of n-SASL as a function of the doxyl group position (n) along the hydrocarbon chain in DMPC (A) and EYPC (B) bilayers, with (○) and without (●) 10 mol% FB_1 at 37°C. S was calculated from the ESR spectra by using the equation $S = 0.5407(T_{\parallel} - 2T_{\perp})/a$ where $a = (T_{\parallel} + 2T_{\perp})/3$. $T_{\parallel}$ and $T_{\perp}$ were measured from the ESR spectra as indicated in Fig. 1.

RESULTS AND DISCUSSION

A typical ESR spectrum obtained from 7-SASL in EYPC membranes at 37°C is displayed in Figure 1. In the membrane, SASL undergo anisotropic rotational motion. The ESR spectra of n-doxylstearic acid spin labeled membranes were characterized by calculating the values of the order parameter S (29,30). As indicated in Figures 2A and 2B, FB_1 perturbs the hydrocarbon chains of both DMPC and EYPC membranes. At 37°C, FB_1 decreases the order parameter S, more for 5- and 7-SASL (which are positioned closer to the surface of the membrane) than for 12- or 16-SASL (near the center of the membrane bilayer). Also, FB_1 shows slight changes in S value on 10-SASL in both DMPC and EYPC. As depicted in Fig. 3, the ESR spectrum of 16-SASL in DMPC at 37°C displays three lines that are more narrow than those of Fig. 1, which indicates that the spin label is in fast motion near the membrane center. The effective rotation correlation times τ is

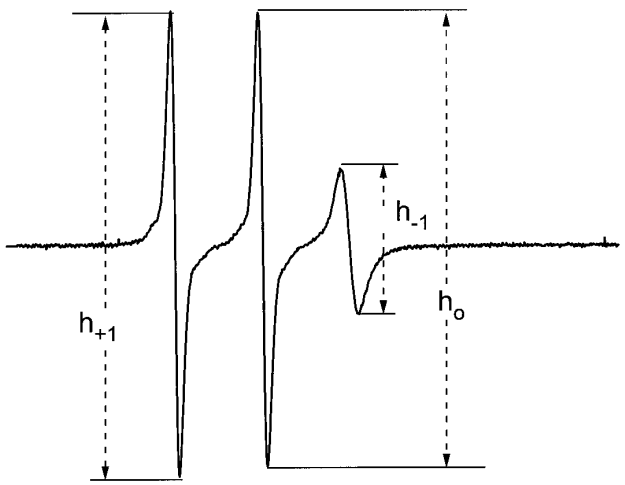

FIG. 3. ESR spectrum of 16-SASL in DMPC at 37°C in 0.1 M Hepes buffer, pH 7.2. The rotation correlation time was calculated from the ESR spectra by using the equation $\tau = 6.51 \times 10^{-10}\ \Delta H_o[(h_o/h_{-1})^{1/2} - (h_o/h_{+1})^{1/2}]$ s. ΔH_o is the center line width, h_o, h_{-1} and h_{+1} were measured from the ESR spectra as indicated in the figure.

an another empirical ESR parameter that is often used to characterize the dynamics of 12- and 16-SASL in membranes (31). In the presence of 0 and 10 mol% FB_1, the τ values for 16-SASL in DMPC at 37°C are 0.79 and 0.80, respectively; similar values in EYPC of 0.78 and 0.77 nS were obtained. Likewise, for 12-SASL in DMPC and EYPC membranes, no apparent effects of FB_1 were observed (data not shown). In essence, FB_1 perturbs the hydrocarbon chain relatively close to the membrane surface in fluid phase membranes and has minimum effect near the membrane center.

Because bimolecular collisions of molecular oxygen and nitroxide induce Heisenberg spin exchange that yields a broadening of ESR lines (9), the changes in ESR line width H(x) are proportional to the oxygen diffusion-concentration product (10), which was used as an experimental parameter to assess the oxygen transport properties (or oxygen permeability) of fluid phase membranes. The ESR line width changes were measured at 6 different depths in the membranes (with and without FB_1), which provide profiles (32) of oxygen transport across membranes, as depicted in Figs. 4A and 4B; the values of the line width change near the surface region of the bilayer are *ca.* 30% higher with 10 mol% FB_1, compared to the controls. There are also weak effects of FB_1 on these values near the center of the membranes. A comparison of the DMPC and EYPC oxygen transport profiles indicates that FB_1 affects the oxygen transport in both investigated membranes in a similar way.

In conclusion, fumonisin B_1 perturbs a complex interrelationship between membrane structure (vis-a-vis S and τ) and oxygen permeability; at physiological temperatures, *i.e.*, in the fluid phase, the region near the surface of the membrane is disrupted and displays a pronounced increase in oxygen permeability for both DMPC and EYPC.

Analysis of the physical properties of the fumonisins in lipid environments may be critical to unraveling the fundamental bioactivities of this family of mycotoxins. Because the rates of oxygen-mediated reactions depend, in part, on the collision frequencies between oxygen and substrate, the effects of FB_1 on oxygen permeability in membranes may bear vital biological implications, *e.g.*, reactions involving active forms of oxygen, such as lipid peroxidation. The *in vivo* implications, such as atherogenic potential, remain to be determined.

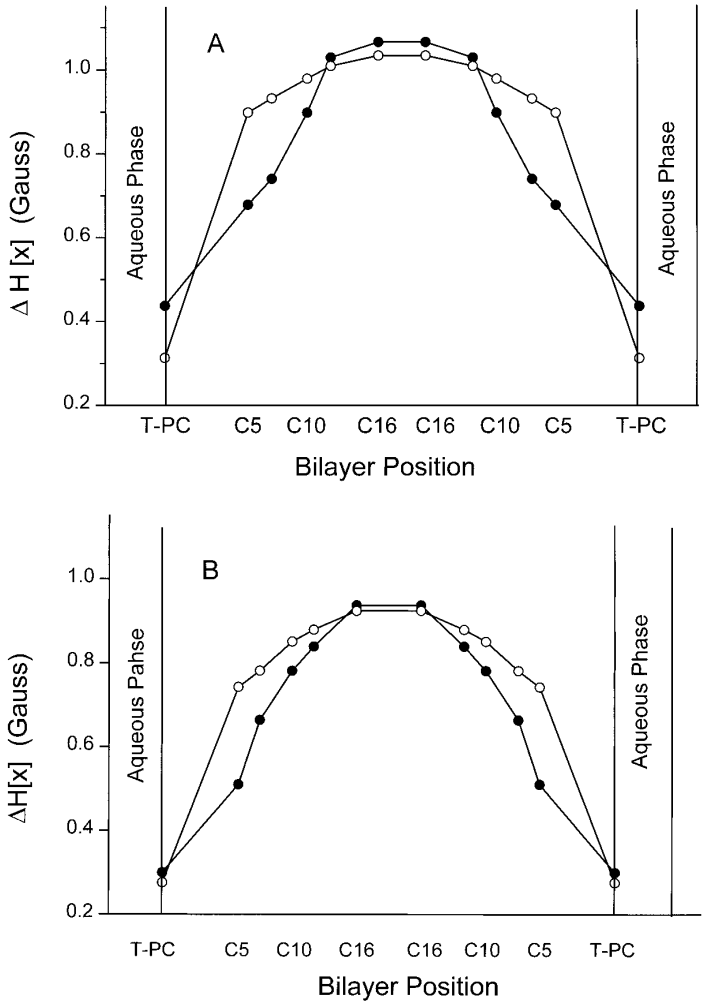

FIG. 4. Profiles of the oxygen ESR line broadening (or relative oxygen permeability) across DMPC (A) and EYPC (B) membranes, with (○) and without (●) 10 mol% FB_1 at 37°C. The abscissa indicates the approximate depth in the membrane bilayers at which each spin label is positioned (T-PC represents Tempocholine-PC). $\Delta H(x) = H_{pp}(x, O_2) - H_{pp}(x, N_2)$, where $H_{pp}(x, O_2)$ and $H_{pp}(x, N_2)$ are the peak-to-peak ESR line widths of the first derivative spectrum, measured at the center line position for samples saturated with oxygen and nitrogen, respectively.

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