Curcumin is a Modulator of Bilayer Material Properties†

Helgi I. Ingolfsson,‡,§ Roger E. Koeppe II,|| and Olaf S. Andersen*,§

Cornell/Rockefeller/Sloan-Kettering Tri-Institutional Program in Computational Biology & Medicine, New York, New York 10065, Department of Physiology and Biophysics, Weill Cornell Medical College, New York, New York 10065, Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, Arkansas 72701

Received May 24, 2007; Revised Manuscript Received June 27, 2007

ABSTRACT: Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is the major bioactive compound in turmeric (*Curcuma longa*) with antioxidant, antiinflammatory, anticarcinogenic, and antimutagenic effects. At low μM concentrations, curcumin modulates many structurally and functionally unrelated proteins, including membrane proteins. Because the cell membranes' lipid bilayer serves as a gate-keeper and regulator of many cell functions, we explored whether curcumin modifies general bilayer properties using channels formed by gramicidin A (gA). gA channels form when two monomers from opposing monolayers associate to form a conducting dimer with a hydrophobic length that is less than the bilayer hydrophobic thickness; gA channel formation thus causes a local bilayer thinning. The energetic cost of this bilayer deformation alters the gA monomer ↔ dimer equilibrium, which makes the channels' appearance rate and lifetime sensitive to changes in bilayer material properties, and the gA channels become probes for changes in bilayer properties. Curcumin decreases bilayer stiffness, increasing both gA channel lifetimes and appearance rates, meaning that the energetic cost of the gA-induced bilayer deformation is reduced. These results show that curcumin may exert some of its effects on a diverse range of membrane proteins through a bilayer-mediated mechanism.

Turmeric has a long history in Asian cooking and traditional Indian Ayurvedic medicine (*1*−*3*). The major active ingredient in turmeric is the polyphenol curcumin (diferuloylmethane, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), Figure 1.

Curcumin has been reported to have a wide range of pharmacological effects, including antioxidant, antiinflammatory, anticarcinogenic, and antimutagenic actions (*4*−*7*). It is not clear how curcumin exerts these many different effects, nor is it clear whether curcumin exerts its effects through just one or through several mechanisms.

Indeed, curcumin modulates the function and expression of a wide range of unrelated proteins (*5*, *6*) including transcription factors (e.g., NF-κB, PPARγ, AP-1, and STAT3); antiapoptotic proteins (e.g., cytochrome *c*, caspase 9, and Bcl-2); and membrane proteins such as ion pumps, receptors, and channels (see Table 1).

To date no curcumin binding site seems to have been identified on any of these "target" proteins, but curcumin accumulates in biological membranes and is a known membrane-destabilizing agent at concentrations of ∼100 μM (*25*, *26*). These observations raise the possibility that curcumin at the low μM concentrations used in most of the studies in Table 1 could alter membrane protein function by modulating the host lipid bilayer properties, a mechanism whose feasibility we explore in this article.

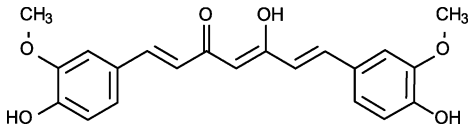

FIGURE 1: Structure of curcumin (diferuloylmethane).

Generally, bilayer-mediated alterations of membrane protein function arise because membrane-associated proteins are coupled to the bilayer through hydrophobic interactions. This hydrophobic coupling allows changes in bilayer properties to propagate to the proteins, and vice versa (*27*). Specifically, if membrane proteins undergo conformational changes that involve the protein/bilayer boundary, the protein conformational changes will perturb the adjacent bilayer, which would make the proteins' conformational or state preference sensitive to changes in bilayer material properties (*28*−*31*).

To explore whether curcumin alters lipid bilayer properties at pharmacologically relevant concentrations, we used gramicidin (gA[1]) channels to probe for curcumin-induced changes in bilayer material properties (thickness, lipid intrinsic curvature, and the bilayer compression and bending moduli).

† This work was supported by NIH Grants GM021342 (O.S.A.) and RR15569 (R.E.K.) and the Cornell/Sloan-Kettering Program in Computational Biomedicine (H.I.I.).

* Address correspondence to this author: Dept. Physiology and Biophysics, Weill Medical College of Cornell University, C-501B, 1300 York Avenue, New York, NY 10065. Tel: (212) 746-6350. Fax: (212) 746-6350. E-mail: sparre@med.cornell.edu.

‡ Cornell/Rockefeller/Sloan-Kettering Tri-Institutional Program in Computational Biology & Medicine.

§ Weill Cornell Medical College.

|| University of Arkansas.

[1] Abbreviations: DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; gA, gramicidin; AgA(15), gramicidin analogue [Ala[1]]gA; gA$^-$(13), gramicidin analogue des-[Val[1]-Gly[2]]gA$^-$; CFTR, cystic fibrosis transmembrane conductance regulator; curc, used in the context of curcumin concentration [curc], surface density {curc}, and mole mole-fraction m_{curc}.

10.1021/bi701013n CCC: $37.00
Published on Web 08/17/2007

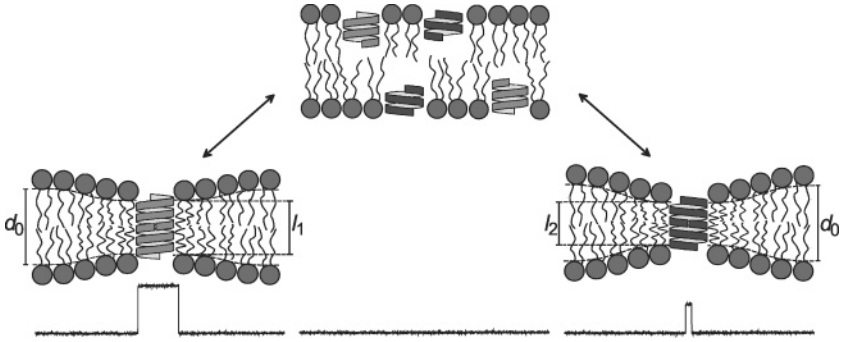

FIGURE 2: Schematic model of gA channel formation in lipid bilayers. The three panels show unassociated monomers (top, middle), a right-handed AgA(15) dimer (left), and a left-handed gA^-(13) dimer (right). The characteristic electrophysiological signatures associated with each state are shown at the bottom of the figure. The hydrophobic thickness of the bilayer is denoted d_0, and the hydrophobic length of the gramicidin channels is l_1 and l_2 for AgA(15) and gA^-(13), respectively. The gA/lipid mole-fraction is $\sim 10^{-6}$ (*47*), so each gA monomer or channel should be independent of any other channel.

Table 1: Membrane Proteins That Are Modified by Curcumin

protein	curcumin activity	refs
epidermal growth factor receptor (EGFR)	suppresses expression, inhibits tyrosine phosphorylation	*8, 9*
human epidermal growth factor receptor 2 (ErbB-2)	promotes degradation	*10*
peroxisome proliferator-activated receptor-γ (PPARγ)	stimulates trans-activating activity	*9*
vascular endothelial growth factor (VEGF)	suppresses expression	*11*
cystic fibrosis transmembrane conductance regulator (CFTR)	prolongs channel open time, reducing channel close time	*12*−*15*
inositol 1,4,5-trisphosphate-sensitive Ca^{2+} channel ($InsP_3$ receptor)	inhibitor	*16*
Kv1.4 potassium channels	inhibitor	*17*
mitochondrial permeability transition pore (PTP)	promotes opening	*18, 19*
F0F1-ATPase/ATP synthase	inhibitor	*20*
multidrug resistance-linked ATP-binding cassette transporter (ABCG2)	inhibitor	*21*
multidrug resistance protein 1 (ABCC1)	inhibitor	*22*
P-glycoprotein (Pgp)	suppresses expression, inhibitor	*23*
sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA)	inhibitor	*24*

gA channels are suitable for this purpose because, first, the channels are formed by the transbilayer dimerization of two nonconducting monomers (*32*); second, the channels' hydrophobic length usually is less than the bilayer's hydrophobic thickness; and third, the bilayer deforms (thins) locally to adjust to the length of the channel (*33*); see Figure 2.

This membrane deformation incurs an energetic cost that depends on bilayer material properties, which in turn are reflected in the gA channel appearance rate and average lifetime, thus allowing the channels to function as bilayer-spanning force transducers (*34*). This feature allows for the detection of curcumin-induced alterations in bilayer material properties, whether curcumin increases or decreases bilayer stiffness (*35*). A decrease in stiffness causes an increased gA channel appearance rate and lifetime; conversely, an increase in stiffness causes a decreased gA channel appearance rate and lifetime.

It is possible to extend this analysis by noting that the energetic cost of deforming the bilayer, ΔG^0_{def}, can be expressed as (*36*)

$$\Delta G^0_{def} = H_B(d_0 - l)^2 + H_X(d_0 - l)c_0 + H_C c_0^2 \quad (1)$$

The local bilayer thinning associated with channel formation thus will result in a disjoining force (F_{dis}) that the bilayer imposes on the bilayer-spanning channel, tending to pull it apart (*27*):[2]

$$F_{dis} = 2H_B(d_0 - l) + H_X c_0 \quad (2)$$

where the coefficients H_B, H_X, and H_C are determined by the bilayer elastic moduli and thickness together with the channel geometry (*36*), d_0 is the bilayer's hydrophobic thickness, l is the channel's hydrophobic length, and c_0 is the lipid intrinsic curvature. Equations 1 and 2, and the underlying theory of elastic bilayer deformations (*37*, *38*), have been tested and calibrated by examining how the gA channel lifetimes vary as a function of lipid bilayer thickness (*39*).

It is, in principle, possible to distinguish between changes in the bilayer elastic moduli and intrinsic curvature by using gA analogues of different length, which will result in the formation of channels of different hydrophobic length (with different channel−bilayer hydrophobic mismatches, $d_0 - l$). Changes in intrinsic curvature would alter the lifetimes of short and long channels equally, whereas changes in elastic moduli (in H_B and H_X) would result in a greater change in F_{dis} for the channel with the greater hydrophobic mismatch.

Using this approach we find that curcumin—at the concentrations at which it modifies many membrane proteins—is a modifier of lipid bilayer material properties. Curcumin thus joins an increasing number of small bioactive phytochemicals (*35*, *40*, *41*) that decrease bilayer stiffness and alter membrane protein function. The present results thus provide for a possible mechanistic basis for curcumin's modification of membrane protein function.

[2] The deformation energy, ΔG^0_{def}, is determined from the local bilayer compression and bending contributions (*36*, *37*). When solving this integral for the energy, the result can be expressed in the form of eq 1. The disjoining force is then obtained as the partial derivative of ΔG^0_{def} with respect to the hydrophobic mismatch ($d_0 - l$).

EXPERIMENTAL PROCEDURES

1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) from Avanti Polar Lipids (Alabaster, AL) was used without further purification. The bilayer-forming solutions were 2% (w/v) of DOPC in 99.9% pure *n*-decane from ChemSampCo (Trenton, NJ). Curcumin was from Sigma Chemical Co. (St. Louis, MO) and was dissolved in dimethyl sulfoxide from Burdick & Jackson (Muskegon, MI). Curcumin is susceptible to hydrolytic degradation in electrolyte solutions, especially at high pH (*42*), and is sensitive to light (*43*, *44*). To minimize curcumin deterioration, individual samples were prepared for each day of experiments and kept at −40 °C; exposure to light was kept to a minimum. Because curcumin absorbs to plastics, care was taken to minimize contact with plastic materials—though the single-channel experiments were done in Teflon chambers, which resulted in a loss of 15−30% of added curcumin (data not shown). The gramicidin analogues [Ala^1]gA (AgA(15)) and des-[Val^1-Gly^2]gA$^-$ (gA$^-$(13)) were synthesized and purified as described previously (*45*).

Gramicidin single-channel experiments were done at 25 ± 1 °C using the bilayer punch method (*46*). The electrolyte solution was 1 M NaCl, buffered to pH 7.0 using 10 mM HEPES (Sigma). The single-channel measurements where done using a Dagan 3900A Integrating patch clamp (Minneapolis, MN), with 100 or 200 mV applied potential. For some experiments, the reported gA channel lifetimes were pooled from data recorded at both 100 and 200 mV applied potential. In 1.0 M NaCl, the lifetimes of AgA(15) and gA$^-$(13) channels vary less than 5% from experiment to experiment and are insensitive to voltage, with no systematic variation between 100 and 200 mV (results not shown). The current signal was low-pass filtered at 2 kHz, digitized at 20 kHz, and digitally filtered at 500 Hz. Single-channel current transition amplitudes and lifetimes were determined and the respective histograms constructed as described previously (*46*, *47*). Average channel lifetimes (τ) were determined by fitting a single exponential distribution $N(t)/N(0) = \exp\{-t/\tau\}$, where $N(t)$ is the number of channels with lifetime longer than time t (*47*), to the lifetime histograms.

The membrane capacitance was measured using a sawtooth potential waveform (*31*).

Curcumin's stability and adsorption to Teflon were determined using 25 μM curcumin and the same electrolyte solution as was used in the single-channel experiments. Measurements were taken with a Spectronic AquaMate spectrophotometer (Thermo Electron Corporation, Waltham, MA). The absorption spectra were scanned from 300 to 500 nm every 10−15 min; all comparisons were done using the absorption peak of 426 nm (*48*). For the curcumin stability measurements, comparisons were made between samples without or with exposure to light; for the adsorption measurements, comparisons were made between samples kept in a glass beaker and samples kept in Teflon chambers.

Curcumin's lipid bilayer/electrolyte partition coefficient (K_p) was determined from the difference between the second derivatives of the absorption spectra measured in solutions having increasing concentrations of unilamellar lipid vesicles (*49*). The vesicles were prepared by sonication and miniextrusion (*50*), using a 0.1 μm filter (Avanti Polar Lipids). The lipid suspension was passed though the filter 11 times, at room temperature, which gives a vesicle size distribution with 60−100 nm average diameter (*50*, *51*). Absorption spectra were collected between 300 and 500 nm (at 25 ± 1 °C) with 25 μM curcumin in the electrolyte solution and varying concentrations of lipid vesicles. Actual phospholipid concentrations were assayed following Chen et al. (*52*).

K_p was determined as described by Kitamura et al. (*49*). In brief a nonlinear least-square fit of

$$\Delta D = \frac{\Delta D_{max} K_p [L]}{[W] + K_p [L]} \quad (3)$$

to the results was done using MATLAB 7 (The MathWorks, Inc, Natick, MA), where [W] and [L] are molar water and lipid concentrations, respectively, and ΔD is the difference between the second derivatives of the baseline (no phospholipid) spectrum and spectra measured at increasing phospholipid concentrations (24, 41, 63, 84, 130, 185, 276, 369, and 573 μM; the individual determinations have less than 2% error, as judged by repeated assays). The second derivatives were obtained from a Savizky−Golay fit to the absorption spectra, and ΔD was determined at the wavelength that gives maximal signal, in this case at $\lambda = 423$ nm. ΔD_{max} and K_p are fitted parameters with ΔD_{max} being the estimated maximal ΔD. The standard deviation of the K_p estimate was determined using the leave-one-out jackknife (*53*).

RESULTS

Curcumin is a potent modifier of bilayer material properties, as evidenced by the curcumin-induced changes in gA channel function, whether it be the longer, right-handed AgA(15) channels or the shorter, left-handed gA$^-$(13) channels. At low μM concentrations, curcumin increases both the appearance rates and lifetimes of both channel types as already evident from the single-channel current traces (Figure 3).

Curcumin does not alter the current transition amplitudes, as illustrated in the current transition amplitude histograms (Figure 4A), at concentrations up to 6 μM (Figure 5A).

In contrast to the invariant current transition amplitudes, the lifetimes of both gA channel types increase as a function of the curcumin concentration ([curc]) (Figures 4B and 5B). Importantly, the lifetime of the shorter gA$^-$(13) channels increases more than the lifetime of the longer AgA(15) channels (Figure 5B). This result shows that the effect of curcumin varies as a function of the channel−bilayer hydrophobic mismatch. That is, curcumin reduces the $2H_B(d_0 - l)$ term in eq 2, most likely by reducing the elastic coefficient H_B, though we cannot exclude that curcumin may also alter the intrinsic curvature (see Discussion).

For a given channel length, the disjoining force also varies as a function of bilayer thickness (cf. eq 2), meaning that the curcumin-induced increases in channel lifetime could result from bilayer-thinning. Any changes in bilayer thickness, however, would be expected to affect long and short channels equally and therefore be unable to account for the results in Figure 5B. To probe whether curcumin thins the bilayer, we measured the membrane capacitance in the absence and presence of 10 μM curcumin. The capacitance

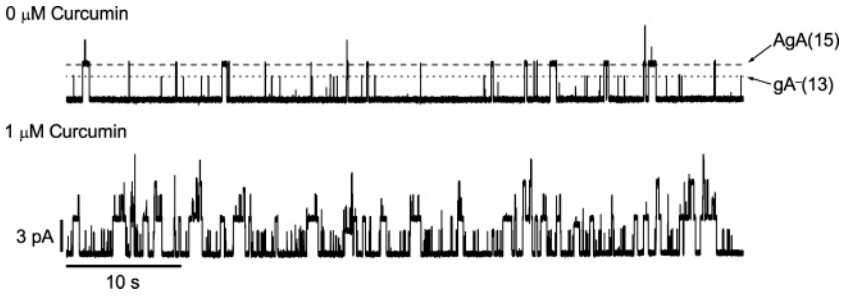

FIGURE 3: Curcumin enhances gA channel activity. Single-channel current traces recorded before and after addition of 1 μM curcumin to a DOPC/*n*-decane bilayer that has been doped with gA$^-$(13) and AgA(15). The two horizontal lines are drawn at the mean single channel current amplitude for the gA$^-$(13) channels (dotted) and the AgA(15) channels (dashed). 1.0 M NaCl, 10 mM HEPES, pH 7.0, 25 °C, +200 mV, 500 Hz.

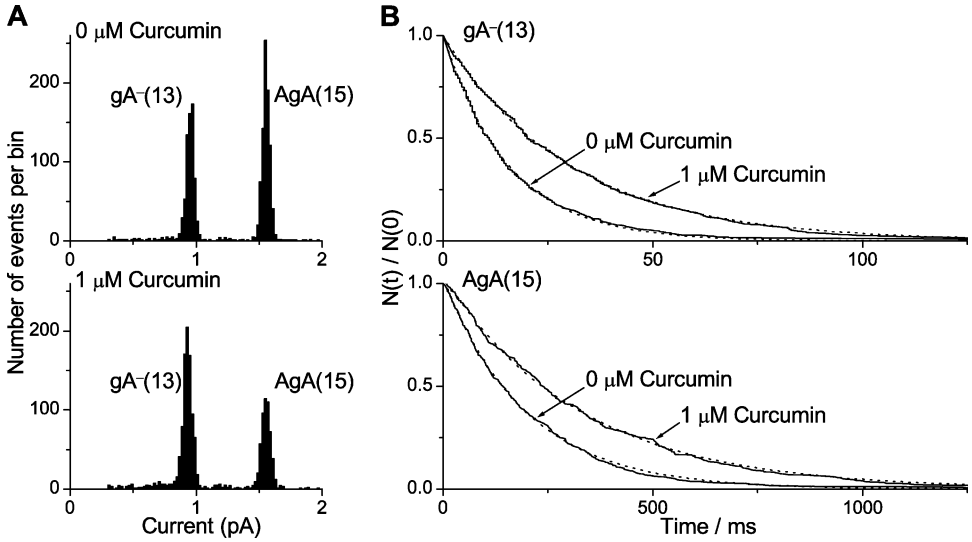

FIGURE 4: Effects of curcumin on gA channel current transitions and lifetimes. A. Current transition amplitude histograms before and after addition of 1 μM curcumin for gA$^-$(13) channels (left peak) and AgA(15) channels (right peak). B. Normalized single-channel survivor histograms for gA$^-$(13) channels (top) and AgA(15) channels (bottom). Note the 10-fold difference in time scale in the lifetime histograms for the shorter gA$^-$(13) channels and the longer AgA(15) channels. The histograms were fitted with a single exponential distribution (dotted line) given by $N(t)/N(0) = \exp\{-t/\tau\}$. 1.0 M NaCl, 10 mM HEPES pH 7.0, 25 °C, ±100 mV, 500 Hz.

was 4.8 ± 0.4 nF/mm^2 in the absence and 4.4 ± 0.2 nF/mm^2 in the presence of 10 μM curcumin (mean ± SD, n = 5). Curcumin does not decrease bilayer thickness; if anything it increases the thickness.

The single-channel experiments were complicated by the curcumin coloration of the electrolyte solution. Beyond 6 μM curcumin, the lack of visibility hinders the experiment. Moreover curcumin adsorbs avidly to lipid bilayers (present results) and is a bilayer destabilizing agent (*25*, *26*). At concentrations ≥3 μM, curcumin reduces the stability of the bilayer, meaning that the membranes break frequently and need to be re-formed. Each time a new membrane is formed, a small amount of DOPC/*n*-decane solution is added to the system, which will tend to deplete the aqueous solutions of curcumin. Thus, if a new membrane is formed in the presence of curcumin, the resulting decrease in [curc] will cause a reduction in the curcumin-induced effect on gA channel function (by 20−50%, as determined by measuring the lifetime before and after breaking and re-forming a membrane). To understand better the basis for the observed decrease in curcumin's apparent effect, we determined curcumin's water/bilayer partition coefficient (K_p), which was 6.3 ± 1.4 × 10^5 (Figure 6).

Given K_p, we can estimate the curcumin adsorption to the bilayer following Bruno et al. (*54*). Let $\{\text{curc}\}_m$ denote the [curc] in each leaflet of the membrane phase (in moles/area) and $[\text{curc}]_a$ the aqueous [curc]:

$$\{\text{curc}\}_m = K_p \frac{d_0}{2}[\text{curc}]_a \tag{4}$$

where d_0 is the bilayer thickness. Knowing K_p, $\{\text{curc}\}_m$, and $[\text{curc}]_a$, the conservation relation for curcumin becomes

$$[\text{curc}]_{nom} V_{aq} = \frac{[\text{curc}]_a V_{aq}}{(1 - F_T)} + [\text{curc}]_m V_{lip} \tag{5}$$

where $[\text{curc}]_{nom}$ is the nominal [curc] added to the aqueous solutions, V_{aq} the aqueous volume, V_{lip} the volume of the lipid forming solution, and F_T the fraction of curcumin that is adsorbed to the walls of the Teflon chamber. Combining eqs 4 and 5:

$$\{\text{curc}\}_m = K_p \frac{d_0}{2}[\text{curc}]_a = \frac{K_p [\text{curc}]_{nom} V_{aq} d_0}{(V_{aq}/(1 - F_T) + K_p V_{lip})2} \tag{6}$$

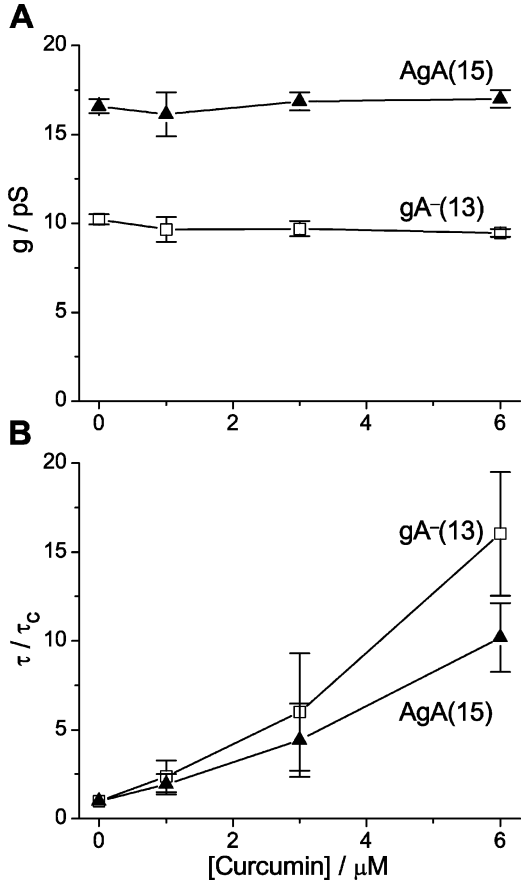

FIGURE 5: gA channel conductance (g) and lifetime (τ) as a function of [curc]. A. AgA(15) and gA$^-$(13) channel conductance (mean ± SE for $n = 3-4$; mean ± range for $n = 2$). B. AgA(15) and gA$^-$(13) channel lifetimes normalized with respect to control (0 μM) (mean ± SE for $n = 3-4$; mean ± range for $n = 2$). 1.0 M NaCl, 10 mM HEPES pH 7.0, 25 °C, ±100−200 mV, 500 Hz.

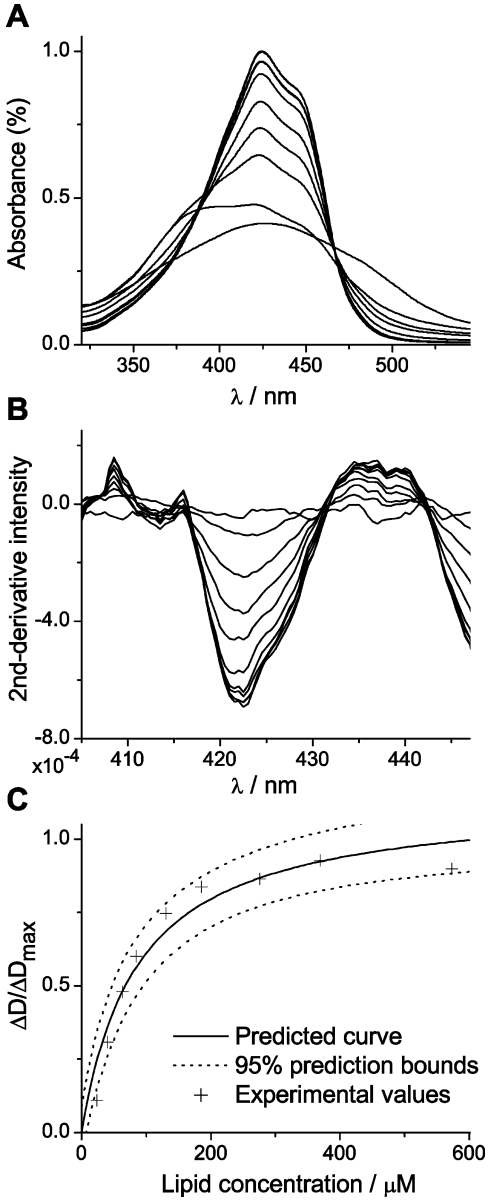

FIGURE 6: Determination of the curcumin's lipid bilayer/electrolyte partition coefficient (K_p). A. Absorbance of curcumin at varying concentration of DOPC liposomes (0, 24, 41, 63, 84, 130, 185, 276, 369, and 573 μM phospholipid); the results are normalized to the peak adsorption in the absence of lipid vesicles. B. Second derivative of absorbance spectra. The trough at 423 nm was used for the analysis in panel C. C. The solid line is a nonlinear least-squares fit of $\Delta D = (\Delta D_{max} K_p[L])/([W] + K_p[L])$ to the data with $K_p = 6.3 \pm 1.4 \times 10^5$; standard deviation was estimated with a leave-one-out jackknife. The dotted lines are upper and lower 95% confidence bounds for a new observation.

In our case $V_{aq} = 5$ mL, $V_{lip} \approx 2$ μL, $F_T \approx 0.3$, and $d_0 \approx 4$ nm, which leads to $[curc]_a \approx (4 \times 10^{-3})[curc]_{nom}$ and $\{curc\}_m \approx (5 \times 10^{-4})[curc]_{nom}$ cm (note the difference in dimensionality: moles/area vs moles/volume). If V_{lip} were 4 μL, however, then $[curc]_a$ and $\{curc\}_m$ would be 2-fold less due to the depletion of curcumin from the aqueous phase!

That is, given curcumin's high K_p, any variation in the amount of lipid in the system will alter the curcumin/lipid ratio in the bilayer, which will be reflected in the variation among different experiments (standard errors in Figure 5B). If, however, we compare the relative lifetime increases observed for gA$^-$(13) channels against the relative lifetime increases observed for AgA(15) channels (measured in the same experiment, meaning the same curcumin/lipid ratio) the results display a tight straight line behavior (Figure 7), with a slope of 1.599 ± 0.036, mean ± SD ($r^2 = 0.993$, $n = 16$).

DISCUSSION

Curcumin is a promiscuous modulator of many different biological functions and has been shown to alter the function of numerous proteins (*5*, *6*), including membrane proteins (Table 1). In this study we show that curcumin modifies the function of gA channels. The present results thus add to the long list of proteins that are affected by curcumin. The results further show that curcumin, in addition to its antioxidant effects, modifies lipid bilayer properties, which provides for a novel mechanism by which curcumin could modify membrane protein function.

We discuss first the curcumin-induced changes in gA channel function. Then we consider the implications for integral membrane proteins, including the effects of curcumin on CFTR.

The changes in gA channel function could be due to specific interactions or to general lipid bilayer-mediated changes in channel function. Curcumin most likely does not bind directly to the gA channels. First, gA channels are miniproteins that are fully imbedded in the lipid bilayer with

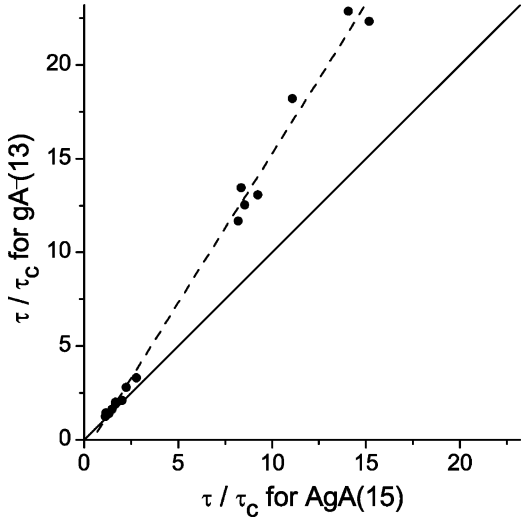

FIGURE 7: The curcumin-induced changes in $gA^-(13)$ channel lifetimes vary as a linear function of the changes in AgA(15) channel lifetimes. Each point denotes the relative change in the lifetime of $gA^-(13)$ channels as a function of the relative change in the lifetime of AgA(15) channels determined in the same experiment. The solid line has a slope of 1 and is drawn for reference, whereas the dashed line is a linear fit to the data giving a slope of 1.599 ± 0.036, mean $\pm$ SD ($r^2 = 0.993$, $n = 16$). 1.0 M NaCl, 10 mM HEPES pH 7.0, 25 °C, ±100−200 mV, 500 Hz.

no obvious binding or catalytic site. Second, curcumin had no effect on the current transition amplitudes for either channel type tested (Figure 5A) which, given the large changes in single-channel conductance when the amino acid sequence is varied (*55*, *56*), makes direct binding unlikely. Third, both the current transition amplitude and lifetime histograms provide evidence for only one population of $gA^-(13)$ channels and one population of AgA(15) channels (Figure 4), with the channel properties varying "continuously" as a function of [curc]. Fourth, curcumin has qualitatively similar effects on the activity of two gA analogues of opposite chirality, except that the changes in channel function were more pronounced for the shorter channels (Figure 5B), similar to what has been observed with other small amphiphiles (*35*, *40*). Fifth, there was no evidence for saturation in the changes in gA channel lifetime as a function of [curc] (Figures 5B and 6), even up to nominal concentrations of 6 μM, where we observed a 15-fold increase in τ.

Curcumin adsorbs avidly to lipid bilayers; in our system the curcumin mole fraction (m_{curc}) in the (unperturbed) bilayer is ~0.01, because curcumin not only will adsorb to the Teflon but also partition into the membrane-forming solution we added to the system. Specifically, for 3 μM curcumin (and $V_{lip} \approx 2$ μL), then the concentration of "free" curcumin in the aqueous solution, $[curc]_a \approx 12$ nM and $\{curc\}_m \approx 1.5 \times 10^{-12}$ moles/cm^2 (or 9×10^{-3} molecules/nm^2); using an area/lipid molecule of ~0.7 nm^2 (*57*), m_{curc} is about 6×10^{-3}. As noted, this estimate applies to the unperturbed bilayer; the local m_{curc} in the vicinity of a channel could be higher due to local accumulation of curcumin adjacent to a bilayer-spanning channel in the perturbed lipid bilayer (*54*).

Though we cannot fully exclude direct curcumin−gramicidin interaction, we conclude that the curcumin-induced changes in gA channel function primarily are due to curcumin's modification of lipid bilayer material properties

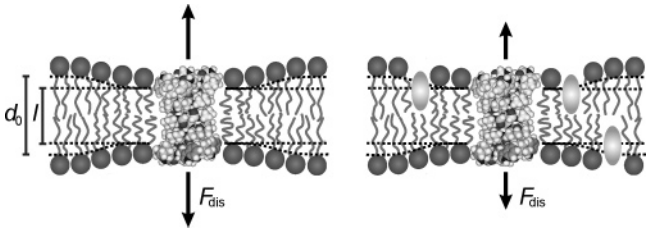

FIGURE 8: Schematic model of curcumin's effect on lipid bilayer properties and gA channel function. When a gA channel forms, the lipid bilayer responds to the local bilayer thinning by imposing a disjoining force (F_{dis}) on the channel. When curcumin adsorbs to the bilayer, the bilayer elastic moduli are reduced, leading to a reduction in the magnitude of F_{dis}, which in turn increases the gA channel lifetime (and appearance rate).

rather than direct curcumin−gramicidin interaction. That is, we can to the first approximation relate the changes in gA channel lifetime to changes in the disjoining force (F_{dis}) the bilayer imposes on the bilayer-spanning channels (cf. eq 2). Because the bilayer thickness is invariant, we conclude that the increase in gA channel lifetimes (Figure 5B) implies that F_{dis} is reduced as [curc] is increased. This reduction in F_{dis} reflects the adsorption of curcumin at the bilayer/solution interface, and the force decreases as m_{curc} increases (Figure 8). The 60% greater lifetime increase for the shorter $gA^-(13)$ channels, relative to the longer AgA(15) channels (Figure 7), further means that the change in F_{dis} is greater for the shorter $gA^-(13)$ channels. The difference in F_{dis} between $gA^-(13)$ and AgA(15) channels reflects the difference in hydrophobic mismatch ($d_0 - l$), and we conclude that curcumin reduces the bilayer elastic moduli, making the bilayer less stiff. Similar amphiphile-dependent changes in bilayer elastic moduli have been reported previously in studies on phospholipid vesicles (*58*−*60*) and in studies using gA channels as force transducers (*35*, *54*).

In the case of integral membrane proteins, it becomes more difficult to exclude that curcumin does not bind to the target protein, cf (*15*, *21*). It is in this context important that unrelated membrane proteins are modified at similar nominal [curc] (but see ref *21*), as this points to a general, physical mechanism rather than a more specific, chemical mechanism. (Though the nominal [curc] usually is in the μM range, the actual aqueous concentrations may be in the nM range; see above). The parsimonious interpretation of these results is that a major effect of curcumin is to alter bilayer elastic moduli and thus the bilayer-dependent contribution to the free energy difference between different membrane protein conformations; cf (*61*). Importantly, such bilayer-mediated mechanisms may show vestiges of specificity, as the relative enrichment (or depletion) of amphiphiles adjacent to a membrane protein in a perturbed bilayer may depend upon the amphiphile structure and the extent of the bilayer perturbation (*54*).

We finally note that curcumin, like capsaicin and genistein, two other small molecules that modify the lipid bilayer material properties (*35*, *40*), also activate CFTR (*62*, *63*) at the concentrations where they modify bilayer material properties. Curcumin has been reported to improve ΔF508-CFTR function in vivo (*12*), a result that could be due to improved trafficking of ΔF508-CFTR to the plasma membrane as well as to enhanced function of channels already in the plasma membrane. Whether or not curcumin improves

trafficking remains controversial (*64*, *65*). That curcumin activates CFTR at the single-channel level is well established (*13*−*15*), although the changes in function depend on the experimental conditions. We find that the effect of curcumin, unlike the effect of capsaicin and genistein, is variable even when using robust gramicidin channels (Figure 5B). This variability could be due to curcumin's high lipid/electrolyte partition coefficient, taken together with curcumin's chemical instability (*42*−44). The variability is reduced by focusing on the curcumin-induced relative lifetime changes for two different gA channels, which eliminates the membrane-to-membrane variation in m_{curc} (Figure 7).

In conclusion, we have shown that curcumin, in addition to its well-established antioxidant effects, is a general modifier of lipid bilayer material properties at the concentration where it modifies the function of many unrelated proteins. Curcumin's modification of bilayer material properties may provide insight into its ability to modify the function of structurally unrelated membrane proteins, by altering the energetic consequences of the hydrophobic coupling between the protein and the host bilayer.

ACKNOWLEDGMENT

We thank Denise V. Greathouse for the synthetic gramicidins and Md. Ashrafuzzaman, Michael J. Bruno, and Jens A. Lundbæk for helpful discussions.

REFERENCES

1. Bodding, P. O. (1983) in *Santal Medicine*, Janasiksha Prochar Kendra, Calcutta, India.
2. Nadkarni, K. M. (1954) in *Indian Materia Medica*, Bopular Book Depot, Bombay.
3. Jain, S. K., and Tarafder, C. R. (1970) Medicinal Plantlore of the Santals (A review of P. O. Bodding's work), *Econ. Bot. 24*, 241−278.
4. Joe, B., Vijaykumar, M., and Lokesh, B. R. (2004) Biological properties of curcumin-cellular and molecular mechanisms of action, *Crit. Rev. Food Sci. Nutr. 44*, 97−111.
5. Aggarwal, B. B., Kumar, A., Aggarwal, M. S., and Shishodia, S. (2005) Curcumin derived from turmeric (Curcuma longa): a spice for all seasons, in *Phytopharmaceuticals in Cancer Chemoprevention* (Bagchi, D., and Preuss, H. G. e., Eds.) pp 349−387, CRC Press, Boca Raton, FL.
6. Shishodia, S., Sethi, G., and Aggarwal, B. B. (2005) Curcumin: getting back to the roots, *Ann. N.Y. Acad. Sci. 1056*, 206−217.
7. Maheshwari, R. K., Singh, A. K., Gaddipati, J., and Srimal, R. C. (2006) Multiple biological activities of curcumin: a short review, *Life Sci. 78*, 2081−2087.
8. Chen, A., Xu, J., and Johnson, A. C. (2006) Curcumin inhibits human colon cancer cell growth by suppressing gene expression of epidermal growth factor receptor through reducing the activity of the transcription factor Egr-1, *Oncogene 25*, 278−287.
9. Chen, A., and Xu, J. (2005) Activation of PPARγ by curcumin inhibits Moser cell growth and mediates suppression of gene expression of cyclin D1 and EGFR, *Am. J. Physiol.: Gastrointest. Liver Physiol. 288*, G447−G456.
10. Tikhomirov, O., and Carpenter, G. (2001) Caspase-dependent cleavage of ErbB-2 by geldanamycin and staurosporin, *J. Biol. Chem. 276*, 33675−33680.
11. Chen, W. H., Chen, Y., and Cui, G. H. (2005) Effects of TNF-alpha and curcumin on the expression of VEGF in Raji and U937 cells and on angiogenesis in ECV304 cells, *Chin. Med. J.* (*Engl.*) *118*, 2052−2057.
12. Egan, M. E., Pearson, M., Weiner, S. A., Rajendran, V., Rubin, D., Glockner-Pagel, J., Canny, S., Du, K., Lukacs, G. L., and Caplan, M. J. (2004) Curcumin, a major constituent of turmeric, corrects cystic fibrosis defects, *Science 304*, 600−602.
13. Berger, A. L., Randak, C. O., Ostedgaard, L. S., Karp, P. H., Vermeer, D. W., and Welsh, M. J. (2005) Curcumin stimulates cystic fibrosis transmembrane conductance regulator Cl- channel activity, *J. Biol. Chem. 280*, 5221−5226.
14. Wang, W., Li, G., Clancy, J. P., and Kirk, K. L. (2005) Activating cystic fibrosis transmembrane conductance regulator channels with pore blocker analogs, *J. Biol. Chem. 280*, 23622−23630.
15. Wang, W., Bernard, K., Li, G., and Kirk, K. L. (2007) Curcumin opens cystic fibrosis transmembrane conductance regulator channels by a novel mechanism that requires neither ATP binding nor dimerization of the nucleotide-binding domains, *J. Biol. Chem. 282*, 4533−4544.
16. Dyer, J. L., Khan, S. Z., Bilmen, J. G., Hawtin, S. R., Wheatley, M., Javed, M. U., and Michelangeli, F. (2002) Curcumin: a new cell-permeant inhibitor of the inositol 1,4,5-trisphosphate receptor, *Cell Calcium 31*, 45−52.
17. Liu, H., Danthi, S. J., and Enyeart, J. J. (2006) Curcumin potently blocks Kv1.4 potassium channels, *Biochem. Biophys. Res. Commun. 344*, 1161−1165.
18. Morin, D., Barthelemy, S., Zini, R., Labidalle, S., and Tillement, J. P. (2001) Curcumin induces the mitochondrial permeability transition pore mediated by membrane protein thiol oxidation, *FEBS Lett. 495*, 131−136.
19. Ligeret, H., Barthelemy, S., Zini, R., Tillement, J. P., Labidalle, S., and Morin, D. (2004) Effects of curcumin and curcumin derivatives on mitochondrial permeability transition pore, *Free Radical Biol Med. 36*, 919−929.
20. Zheng, J., and Ramirez, V. D. (2000) Inhibition of mitochondrial proton F0F1-ATPase/ATP synthase by polyphenolic phytochemicals, *Br. J. Pharmacol. 130*, 1115−1123.
21. Chearwae, W., Shukla, S., Limtrakul, P., and Ambudkar, S. V. (2006) Modulation of the function of the multidrug resistance-linked ATP-binding cassette transporter ABCG2 by the cancer chemopreventive agent curcumin, *Mol. Cancer Ther. 5*, 1995−2006.
22. Chearwae, W., Wu, C. P., Chu, H. Y., Lee, T. R., Ambudkar, S. V., and Limtrakul, P. (2006) Curcuminoids purified from turmeric powder modulate the function of human multidrug resistance protein 1 (ABCC1), *Cancer Chemother. Pharmacol. 57*, 376−388.
23. Anuchapreeda, S., Leechanachai, P., Smith, M. M., Ambudkar, S. V., and Limtrakul, P. N. (2002) Modulation of P-glycoprotein expression and function by curcumin in multidrug-resistant human KB cells, *Biochem. Pharmacol. 64*, 573−582.
24. Bilmen, J. G., Khan, S. Z., Javed, M. H., and Michelangeli, F. (2001) Inhibition of the SERCA Ca2+ pumps by curcumin. Curcumin putatively stabilizes the interaction between the nucleotide-binding and phosphorylation domains in the absence of ATP, *Eur. J. Biochem. 268*, 6318−6327.
25. Jaruga, E., Salvioli, S., Dobrucki, J., Chrul, S., Bandorowicz-Pikula, J., Sikora, E., Franceschi, C., Cossarizza, A., and Bartosz, G. (1998) Apoptosis-like, reversible changes in plasma membrane asymmetry and permeability, and transient modifications in mitochondrial membrane potential induced by curcumin in rat thymocytes, *FEBS Lett. 433*, 287−293.
26. Jaruga, E., Sokal, A., Chrul, S., and Bartosz, G. (1998) Apoptosis-independent alterations in membrane dynamics induced by curcumin, *Exp. Cell Res. 245*, 303−312.
27. Andersen, O. S., and Koeppe, R. E., II. (2007) Bilayer Thickness and Membrane Protein Function: An Energetic Perspective, *Annu. Rev. Biophys. Biomol. Struct. 36*, 107−130.
28. Andersen, O. S., and Koeppe, R. E., II. (1992) Molecular determinants of channel function, *Physiol. Rev. 72*, S89−S158.
29. Keller, S. L., Bezrukov, S. M., Gruner, S. M., Tate, M. W., Vodyanoy, I., and Parsegian, V. A. (1993) Probability of alamethicin conductance states varies with nonlamellar tendency of bilayer phospholipids, *Biophys. J. 65*, 23−27.
30. Brown, M. F. (1994) Modulation of rhodopsin function by properties of the membrane bilayer, *Chem. Phys. Lipids 73*, 159−180.
31. Lundbæk, J. A., and Andersen, O. S. (1994) Lysophospholipids modulate channel function by altering the mechanical properties of lipid bilayers, *J. Gen. Physiol. 104*, 645−673.
32. O'Connell, A. M., Koeppe, R. E., II, and Andersen, O. S. (1990) Kinetics of gramicidin channel formation in lipid bilayers: transmembrane monomer association, *Science 250*, 1256−1259.
33. Harroun, T. A., Heller, W. T., Weiss, T. M., Yang, L., and Huang, H. W. (1999) Experimental evidence for hydrophobic matching and membrane-mediated interactions in lipid bilayers containing gramicidin, *Biophys. J. 76*, 937−945.

34. Andersen, O. S., Nielsen, C., Maer, A. M., Lundbćk, J. A., Goulian, M., and Koeppe, R. E. 2nd (1999) Ion channels as tools to monitor lipid bilayer-membrane protein interactions: gramicidin channels as molecular force transducers, *Methods Enzymol. 294*, 208−224.
35. Lundbæk, J. A., Birn, P., Tape, S. E., Toombes, G. E., Søgaard, R., Koeppe, R. E., II, Gruner, S. M., Hansen, A. J., and Andersen, O. S. (2005) Capsaicin regulates voltage-dependent sodium channels by altering lipid bilayer elasticity, *Mol. Pharmacol. 68*, 680−689.
36. Nielsen, C., and Andersen, O. S. (2000) Inclusion-induced bilayer deformations: effects of monolayer equilibrium curvature, *Biophys. J. 79*, 2583−2604.
37. Nielsen, C., Goulian, M., and Andersen, O. S. (1998) Energetics of inclusion-induced bilayer deformations, *Biophys. J. 74*, 1966−1983.
38. Huang, H. W. (1986) Deformation free energy of bilayer membrane and its effect on gramicidin channel lifetime, *Biophys. J. 50*, 1061−1070.
39. Lundbæk, J. A., and Andersen, O. S. (1999) Spring constants for channel-induced lipid bilayer deformations. Estimates using gramicidin channels, *Biophys. J. 76*, 889−895.
40. Hwang, T. C., Koeppe, R. E., II, and Andersen, O. S. (2003) Genistein can modulate channel function by a phosphorylation-independent mechanism: importance of hydrophobic mismatch and bilayer mechanics, *Biochemistry 42*, 13646−13658.
41. Adachi, S., Nagao, T., Ingolfsson, H. I., Maxfield, F. R., Andersen, O. S., Kopelovich, L., and Weinstein, I. B. (2007) The Inhibitory Effect of (−)-Epigallocatechin Gallate (EGCG) on Activation of the Epidermal Growth Factor Receptor is Associated With Altered Lipid Order in HT29 Colon Cancer Cells, *Cancer Res. 67*, 6493−6501.
42. Tønnesen, H. H., and Karlsen, J. (1985) Studies on curcumin and curcuminoids. VI. Kinetics of curcumin degradation in aqueous solution, *Z. Lebensm.-Unters. Forsch. 180*, 402−404.
43. Tønnesen, H. H., Karlsen, J., and van Henegouwen, G. B. (1986) Studies on curcumin and curcuminoids. VIII. Photochemical stability of curcumin, *Z. Lebensm.-Unters. Forsch. 183*, 116−122.
44. Tønnesen, H. H. (2002) Solubility, chemical and photochemical stability of curcumin in surfactant solutions. Studies of curcumin and curcuminoids, XXVIII, *Pharmazie 57*, 820−824.
45. Greathouse, D. V., Koeppe, R. E., II, Providence, L. L., Shobana, S., and Andersen, O. S. (1999) Design and characterization of gramicidin channels, *Methods Enzymol. 294*, 525−550.
46. Andersen, O. S. (1983) Ion movement through gramicidin A channels. Single-channel measurements at very high potentials, *Biophys. J. 41*, 119−133.
47. Durkin, J. T., Koeppe, R. E., II, and Andersen, O. S. (1990) Energetics of gramicidin hybrid channel formation as a test for structural equivalence. Side-chain substitutions in the native sequence, *J. Mol. Biol. 211*, 221−234.
48. Oetari, S., Sudibyo, M., Commandeur, J. N., Samhoedi, R., and Vermeulen, N. P. (1996) Effects of curcumin on cytochrome P450 and glutathione S-transferase activities in rat liver, *Biochem. Pharmacol. 51*, 39−45.
49. Kitamura, K., Imayoshi, N., Goto, T., Shiro, H., Mano, T., and Nakai, Y. (1995) Second derivative spectrophotometric determination of partition coefficients of chlorpromazine and promazine between lecithin bilayer vesicles and water, *Anal. Chim. Acta 304*, 101−106.
50. Hope, M. J., Bally, M. B., Webb, G., and Cullis, P. R. (1985) Production of large unilamellar vesicles by a rapid extrusion procedure. Characterization of size distribution, trapped volume and ability to maintain a membrane potential, *Biochim. Biophys. Acta 812*, 55−65.
51. MacDonald, R. C., MacDonald, R. I., Menco, B. P., Takeshita, K., Subbarao, N. K., and Hu, L. R. (1991) Small-volume extrusion apparatus for preparation of large, unilamellar vesicles, *Biochim. Biophys. Acta 1061*, 297−303.
52. Chen, P. S., Jr., Toribara, T. Y., and Warner, H. (1956) Microdetermination of Phosphorus, *Anal. Chem. 28*, 1756−1758.
53. Miller, R. G. (1974) The jackknife-a review, *Biometrika 61*, 1−15.
54. Bruno, M. J., Koeppe, R. E., II, and Andersen, O. S. (2007) Docosahexaenoic acid alters bilayer elastic properties, *Proc. Natl. Acad. Sci. U.S.A. 104*, 9638−9643.
55. Becker, M. D., Greathouse, D. V., Koeppe, R. E., II, and Andersen, O. S. (1991) Amino acid sequence modulation of gramicidin channel function: effects of tryptophan-to-phenylalanine substitutions on the single-channel conductance and duration, *Biochemistry 30*, 8830−8839.
56. Mattice, G. L., Koeppe, R. E., II, Providence, L. L., and Andersen, O. S. (1995) Stabilizing effect of D-alanine2 in gramicidin channels, *Biochemistry 34*, 6827−6837.
57. Nagle, J. F., and Tristram-Nagle, S. (2000) Structure of lipid bilayers, *Biochim. Biophys. Acta 1469*, 159−195.
58. Evans, E., Rawick, W., and Hofmann, A. F. (1995) Lipid bilayer expansion and mechanical disruption in solutions of water-soluble bile acid, *Falk Symp.* 59−68.
59. Ly, H. V., and Longo, M. L. (2004) The influence of short-chain alcohols on interfacial tension, mechanical properties, area/molecule, and permeability of fluid lipid bilayers, *Biophys. J. 87*, 1013−1033.
60. Zhou, Y., and Raphael, R. M. (2005) Effect of salicylate on the elasticity, bending stiffness, and strength of SOPC membranes, *Biophys. J. 89*, 1789−1801.
61. Ashrafuzzaman, Md., Lampson, M. A., Greathouse, D. V., Koeppe, R. E., II, and Andersen, O. S. (2006) Manipulating lipid bilayer material properties using biologically active amphipathic molecules, *18*, S1235−S1255.
62. Ai, T., Bompadre, S. G., Wang, X., Hu, S., Li, M., and Hwang, T. C. (2004) Capsaicin potentiates wild-type and mutant cystic fibrosis transmembrane conductance regulator chloride-channel currents, *Mol. Pharmacol. 65*, 1415−1426.
63. Illek, B., Fischer, H., and Machen, T. E. (1996) Alternate stimulation of apical CFTR by genistein in epithelia, *Am. J. Physiol. 270*, C265−C275.
64. Dragomir, A., Bjorstad, J., Hjelte, L., and Roomans, G. M. (2004) Curcumin does not stimulate cAMP-mediated chloride transport in cystic fibrosis airway epithelial cells, *Biochem. Biophys. Res. Commun. 322*, 447−451.
65. Song, Y., Sonawane, N. D., Salinas, D., Qian, L., Pedemonte, N., Galietta, L. J., and Verkman, A. S. (2004) Evidence against the rescue of defective DeltaF508-CFTR cellular processing by curcumin in cell culture and mouse models, *J. Biol. Chem. 279*, 40629−40633.

BI701013N