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Capsaicin Fluidifies the Membrane and Localizes Itself near the Lipid–Water Interface

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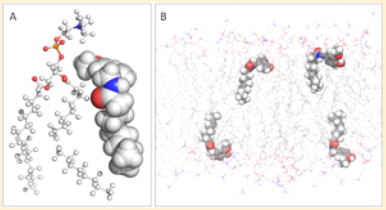
Supporting Information

ABSTRACT: Capsaicin is the chemical responsible for making some peppers spicy hot, but additionally it is used as a pharmaceutical to alleviate different pain conditions. Capsaicin binds to the vanilloid receptor TRPV1, which plays a role in coordinating chemical and physical painful stimuli. A number of reports have also shown that capsaicin inserts in membranes and its capacity to modify them may be part of its molecular mode of action, affecting the activity of other membrane proteins. We have used differential scanning calorimetry, X-ray diffraction, ^{31}P NMR, and ^{2}H NMR spectroscopy to show that capsaicin increases the fluidity and disorder of 1,2-palmitoyl-*sn*-glycero-3-phosphocholine membrane models. By using ^{1}H NOESY MAS NMR based on proton–proton cross-peaks between capsaicin and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine resonances, we determined the location profile of this molecule in a fluid membrane concluding that it occupies the upper part of the phospholipid monolayer, between the lipid–water interface and the double bond of the acyl chain in position *sn*-2. This location explains the disorganization of the membrane of both the lipid–water interface and the hydrophobic palisade.



KEYWORDS: *Capsaicin, membranes, ^{2}H NMR, ^{1}H NOESY MAS NMR, DSC, WAXD, SAXD*

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) is found in many types of peppers corresponding to the genus *Capsicum* and is what makes them spicy hot. In addition to that, capsaicin is widely used as an ingredient in creams of topical use as an analgesic and to treat different medical conditions such as psoriasis. It is known that at submicromolar concentrations capsaicin specifically binds and activates the TPRV1 receptor in sensory nerves, which is responsible for eliciting sensations such as hotness, burning, or pain.[1,2] However, at higher concentrations (micromolar or even millimolar), it may activate signals coming from a wide variety of receptors and membrane proteins, such as the cystic fibrosis transmembrane conductance regulator,[3] the epidermal growth factor receptor,[4] the large conductance calcium-activated potassium channels,[5] airway anion transporters,[6] and the NorA efflux pump,[7] among others. These proteins do not have any evident links among them, and hence the way in which capsaicin interacts with them is not understood. Capsaicin, however, is a lipophilic molecule, and hence its incorporation to membranes should be expected. It has been described in the past that it is able to alter the bilayer membrane.[8,9] In this way, it has been shown that capsaicin regulates voltage-dependent sodium channels by altering lipid bilayer elasticity.[10] In addition to that, capsaicin has an antioxidant role, and it has been suggested that this is exerted by activating the plasma redox system in erythrocytes.[11] The form in which other similar phytochemicals such as curcumin modulate membrane structure has been described in detail,[12−15] and this is also the case for genistein[15] and resveratrol[14,16] and other molecules like the antidepressant desipramine,[17] all of them altering the order of the membrane. These molecules have been shown to affect the activity of membrane proteins.[15,18,19]

Given the importance of capsaicin in pharmacology and in the study of cell signaling, it seems interesting to obtain detailed information about the way in which this molecule modifies the membrane properties and its localization in the bilayer. Following this interest, a very recent molecular dynamics study proposed that capsaicin localizes at the bilayer/solution interface.[15]

In this paper, we have used a combination of physical techniques, such as DSC, ^{2}H NMR, ^{31}P NMR, and X-ray diffraction, to show that capsaicin fluidifies and disorganizes DPPC membranes. In addition, we have used ^{1}H NOESY MAS NMR to show that the polar ring of capsaicin molecules is located close to the lipid–water surface with a bending in the hydrophobic nonenamidyl chain, which will interact with the part of the fatty acyl chains of phospholipids closer to the membrane surface.

RESULTS

Capsaicin Affects the Order of a DPPC Membrane Model. The effect of capsaicin on $L_{\beta'}$ to $P_{\beta'}$ phases and from $P_{\beta'}$ to L_{α} transitions taking place in DPPC membranes was measured by DSC, and the calorimetric profiles of the thermotropic gel-to-liquid-crystalline transition of DPPC (DPPC/DPPC-d_{62}, 1:1

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molar ratio) and capsaicin up to a DPPC/capsaicin 3:1 molar ratio are shown in Figure 1. The pretransition of this DPPC

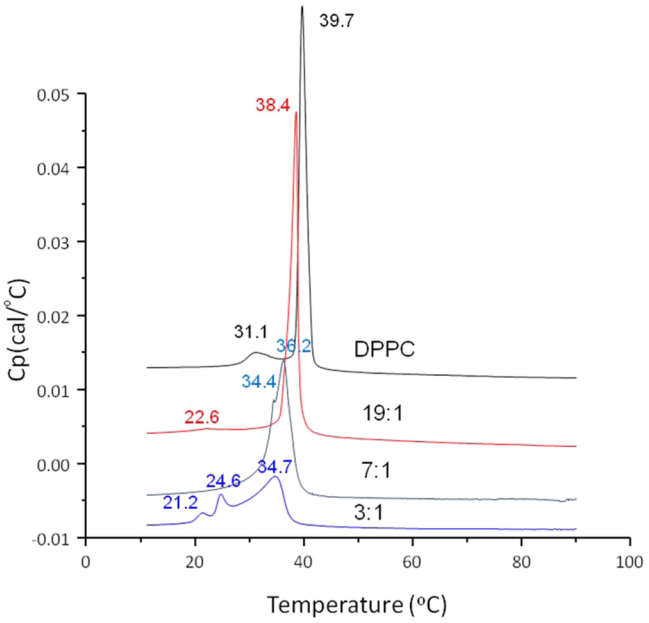

Figure 1. Heating scan thermograms, obtained by using differential scanning for mixtures of DPPC/DPPC-d_{62} (1:1 molar ratio) and capsaicin. The phospholipid/capsaicin molar ratios are given on the traces. Temperatures corresponding to phase transitions are also shown.

started at 29.5 °C, and the onset of the main transition (T_c) at 38.9 °C. The addition of capsaicin to give a DPPC/capsaicin molar ratio of 19:1 shifted and smeared the pretransition that was barely discernible at 22.6 °C. The pretransition totally disappeared at a 7:1 DPPC/capsaicin molar ratio. Furthermore, the presence of increasing concentrations of capsaicin gave place to a progressive reduction in the size of the main transition peak, a broadening of the transition peak and a shift of the T_c transition temperature toward lower temperatures. This behavior reflects a reduction in the order of the membrane induced by the interposition of capsaicin molecules between the acyl chains of DPPC, which disrupts their regular packing in the $L_{\beta'}$ and $P_{\beta'}$ phases. At high concentrations of capsaicin, such as a 3:1 DPPC/capsaicin molar ratio, the transition peak showed at least three components, probably indicating the presence of some more phases, those of them appearing at lower temperatures richer in capsaicin. ΔH values (in kcal/mol) for the main transitions were 8.31 (DPPC), 6.01 (19:1 molar ratio), 5.95 (7:1), and 3.72 (3:1).

Since anionic lipids constitute about 25% of natural membranes DSC measurements were carried out comparing the effect of capsaicin on an unsaturated phospholipid, POPC, and on a mixture POPC/POPG (4:1 molar ratio). In both cases, capsaicin was added to give a phospholipid/capsaicin molar ratio of 5:1. Supplementary Figure 1 shows that the effect of capsaicin was similar to that seen for DPPC, with a broadening of the phase transition and a decrease of the size of the peak, indicating a decrease of ΔH.

Capsaicin Increases the *d*-Spacings in the Gel State of DPPC Membranes. Information on the structural characteristics of DPPC/capsaicin systems was obtained by small-angle X-ray diffraction (SAXD). When phospholipids are organized into multilamellar structures, they give rise to reflections with relative distances of $1:{}^1/_2:{}^1/_3:{}^1/_4:{}^1/_5$. This technique not only defines the macroscopic structure itself but also provides the interlamellar repeat distance in the lamellar phase. The largest first order reflection component corresponds to the interlamellar repeat distance (d value), which is composed of the bilayer thickness and the thickness of the water layer between bilayers.[20] Figure 2A shows the SAXD diffraction patterns for pure DPPC at different temperatures. At 15 °C, the SAXD patterns of pure DPPC showed sharp Bragg reflections such as 65.4:32.6, 21.7, 16.3, and 13.1 Å, up to the fifth order, with spacings related as $1:{}^1/_2:{}^1/_3:{}^1/_4:{}^1/_5$, indicating the existence of multilamellar vesicles. Measurements in the wide angle (WAXD) region provide information about the packing of the phospholipid acyl chains. The wide angle pattern for DPPC at 15 °C (Figure 2B) showed a sharp reflection at 4.21 Å and a broad shoulder at 4.10 Å, indicative of an $L_{\beta'}$ gel phase with hydrocarbon side chains tilted relative to the normal of the bilayer phase and pseudohexagonal chain packing.[21] At 30 °C, the SAXD pattern was essentially the same, reflecting an $L_{\beta'}$ phase in pure DPPC and the same pattern again in WAXD as at 15 °C. At 38 °C, that is, above the pretransition temperature but below the main transition, the SAXD pattern was different with an increase in d-spacing, although keeping the ratios characteristic of a bilayer structure: 73.8:36.5:23.0:16.7 Å. The increase in d-spacing is indicative of a $P_{\beta'}$ structure of pure DPPC at this temperature. Accordingly with this change in structure, the WAXD pattern shows for this temperature a symmetric broad reflection at 4.18 Å. Finally at 45 °C, that is, above the main phase transition, according to DSC, SAXD shows a decrease in d-spacing to 67.2:33.7:21.3, characteristic of bilayer in L_α phase, confirmed by a broad and diffuse spacing in WAXD. All these X-ray results are in very good agreement with previously reported ones.[22]

At 15 °C, the presence of capsaicin induced a progressive increase in the value of the interlamellar repeat distance (Figure 2C–H). The sample with a 19:1 DPPC/capsaicin molar ratio showed a broad peak (Figure 2C,D), and estimating the interlamellar repeat distance from the peak maximum would give 73.5 Å. Spacings at 37.4, 22.9, and 16.2 Å were also detected. It should be underlined that WAXD for this temperature shows a broad and symmetric peak, indicating that the lipid is in a phase with hexagonal packing, unlike the tilted $L_{\beta'}$ present in pure DPPC at this temperature. At 45 °C, both SAXD and WAXD patterns were similar to those observed in pure DPPC. At 15 °C, the DPPC/capsaicin sample with a 7:1 molar ratio (Figure 2E,F) showed a first spacing in the SAXD pattern with a maximum at 74.2 Å and others d-spacings at 37.1 and 23.5 Å, indicating a bilayer structure. As in the previous sample described, the WAXD diffractogram showed a wide and symmetric peak at 4.15 Å, indicative of a phase with hexagonal packing, unlike the tilted $L_{\beta'}$ present in pure DPPC at this temperature. No substantial changes with respect to pure DPPC were observed at 45 °C.

At 15 °C (Figure 2G), the DPPC/capsaicin system with a 3:1 molar ratio showed very wide reflections with a high repeat interlamellar distance of 75.6 Å and a second order reflection at 37.8 Å, indicating the presence of a bilayer but with a loss of macroscopic order. In addition, the WAXD study showed at 15 °C a very broad peak at 4.14 Å (Figure 2H). At 45 °C, the SAXD and WAXD profiles were similar to the samples containing less capsaicin or even pure DPPC.

To test whether this increase in d-spacings was due to an effective increase in the thickness of the bilayer, background-subtracted SAXD patterns for DPPC and DPPC/capsaicin systems in the liquid crystalline phase were analyzed using the global analysis program (GAP). Figure 3 shows the correspond-

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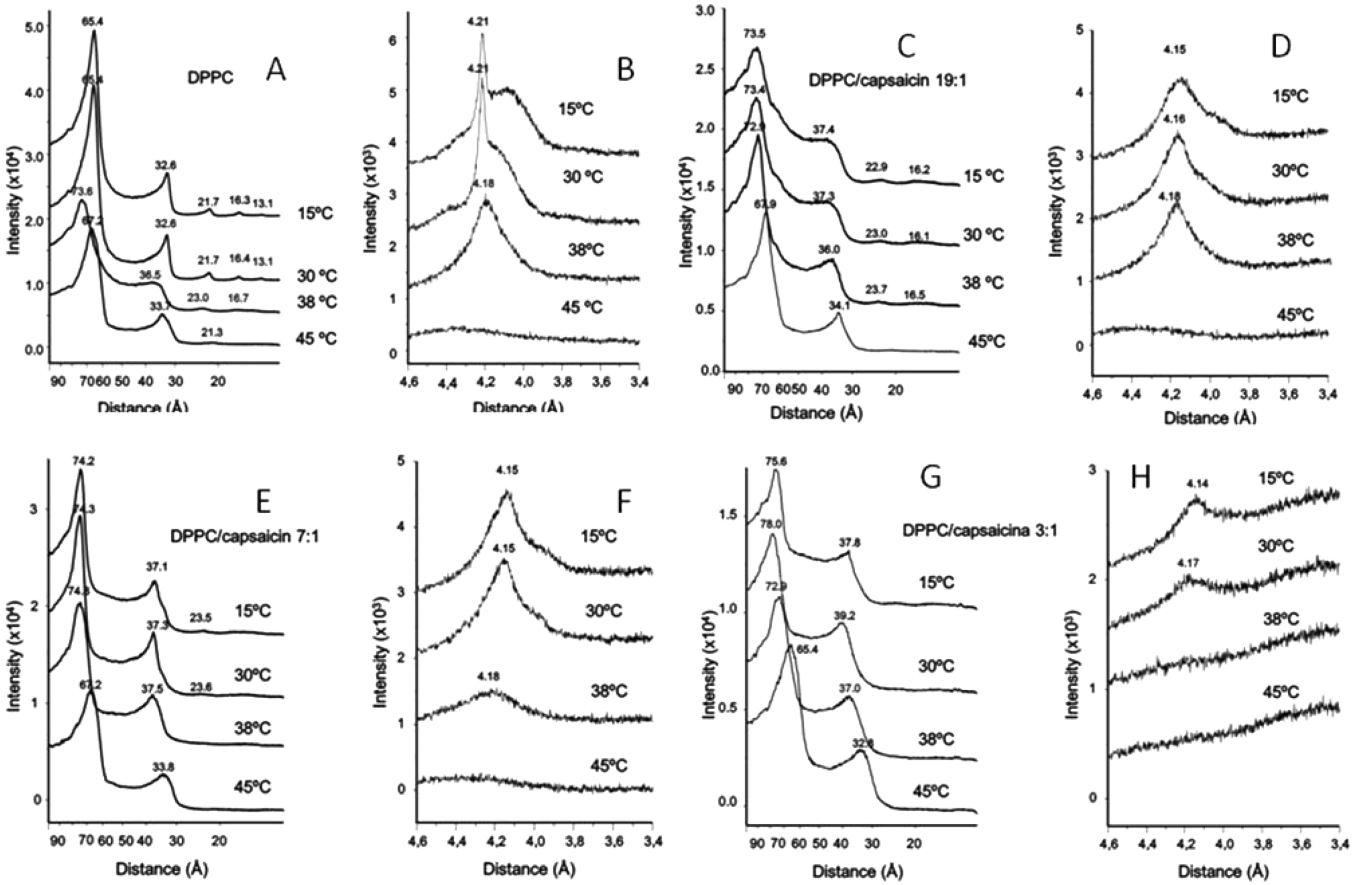

Figure 2. Small angle (A, C, E, and G) and wide angle (B, D, F, and H) X-ray diffraction profiles of DPPC/capsaicin mixtures: Pure DPPC (A, B); DPPC/capsaicin 19:1, molar ratio (C, D); DPPC/capsaicin 7:1, molar ratio (E, F); DPPC/capsaicin 3:1, molar ratio (G, H). Temperatures are shown on the traces. Values corresponding to *d*-spacings in Å are also given on the diffractograms.

ing one-dimensional electron density profiles along the bilayer normal calculated from the SAXD diffraction patterns for DPPC in the absence and in the presence of capsaicin. The d_{p-p} value (d_B or distance between both phosphates of the bilayer) was calculated for the different samples. It can be observed that the presence of capsaicin in the liquid crystalline phase has a negligible effect on membrane thickness, with d_{p-p} = 46.0 Å for pure DPPC and 46.4, 46.6, and 46.4 Å, for the 19:1, 7:1, and 3:1 samples, respectively (see Figure 3), and since only a very small change was observed in the *d*-spacings values at this temperature, it is concluded that in the fluid condition membranes were not changed in either the thickness of the bilayer or the water layer of the multilamellar systems. At 15 °C, however, an increase in the thickness of the membrane was observed to be occasioned by the presence of capsaicin so that whereas pure DPPC presents a d_{p-p} of 42.0 Å, that is increased to 42.9, 45.0, and 46.9 Å for the 19:1, 7:1, and 3:1 samples, respectively. Therefore, the presence of capsaicin in the gel phase really increased the thickness of the membrane, although this is not the case when the membrane is fluid.

^{31}P NMR and ^{2}H NMR Measurements Demonstrate That Capsaicin Disorders DPPC Membranes and Modifies Vesicle Ellipticity. The effect of capsaicin on the phase polymorphism of DPPC was further investigated by ^{31}P NMR spectroscopy. The ^{31}P NMR spectra of aqueous dispersions of DPPC codispersed with different amounts of capsaicin recorded at various temperatures showed patterns with asymmetric line shape with a high-field peak and a low-field shoulder at all temperatures, characteristic of bilayer structure (Supplementary Figure 2). Table 1 shows the chemical shift anisotropy ($\Delta\sigma$) values for the different samples. In the gel state (26 °C), the line shape of pure DPPC is broadened ($\Delta\sigma$ = 68.1 ± 1.0 ppm) compared with the liquid crystalline state (52 °C) where the line shape is considerably narrower ($\Delta\sigma$ = 45.5 ± 1.1 ppm). In the presence of capsaicin, $\Delta\sigma$ values are reduced at all temperatures, so at 26 °C, it went down to 62.8 ± 0.3 ppm in the 19:1 sample, to 51.9 ± 0.8 ppm in the 7:1 one, and to only 42.6 ± 0.5 ppm for the 3:1 sample. It is therefore evident that capsaicin disorders the membrane and that in the case of the 3:1 sample the membrane is already fluid even at 26 °C. Similarly in the case of the 7:1 sample and at 34 °C, $\Delta\sigma$ was 42.7 ppm, indicating a fluid state again. It should be remarked that the spectral pattern obtained at all temperatures and molar ratios DPPC/capsaicin indicate a bilayer structure of the membranes, with no isotropic structures, showing that capsaicin did not induce micellization of the phospholipids at these concentrations (Supplementary Figure 2). Another interesting observation is that the polar headgroup is totally disordered at 26 °C in the 3:1 sample, whereas WAXD (Figure 2) revealed the existence of some order up to 30 °C, which can be interpreted as capsaicin affecting mainly the order at the lipid−water interface and not so much in the core of the membrane.

To get further insight into the effect of capsaicin incorporation on DPPC membranes, we performed ^{2}H NMR experiments. Figure 4A depicts the spectra at different temperatures of a DPPC/DPPC-d_{62} (1:1 molar ratio) membrane from 26 to 48 °C.

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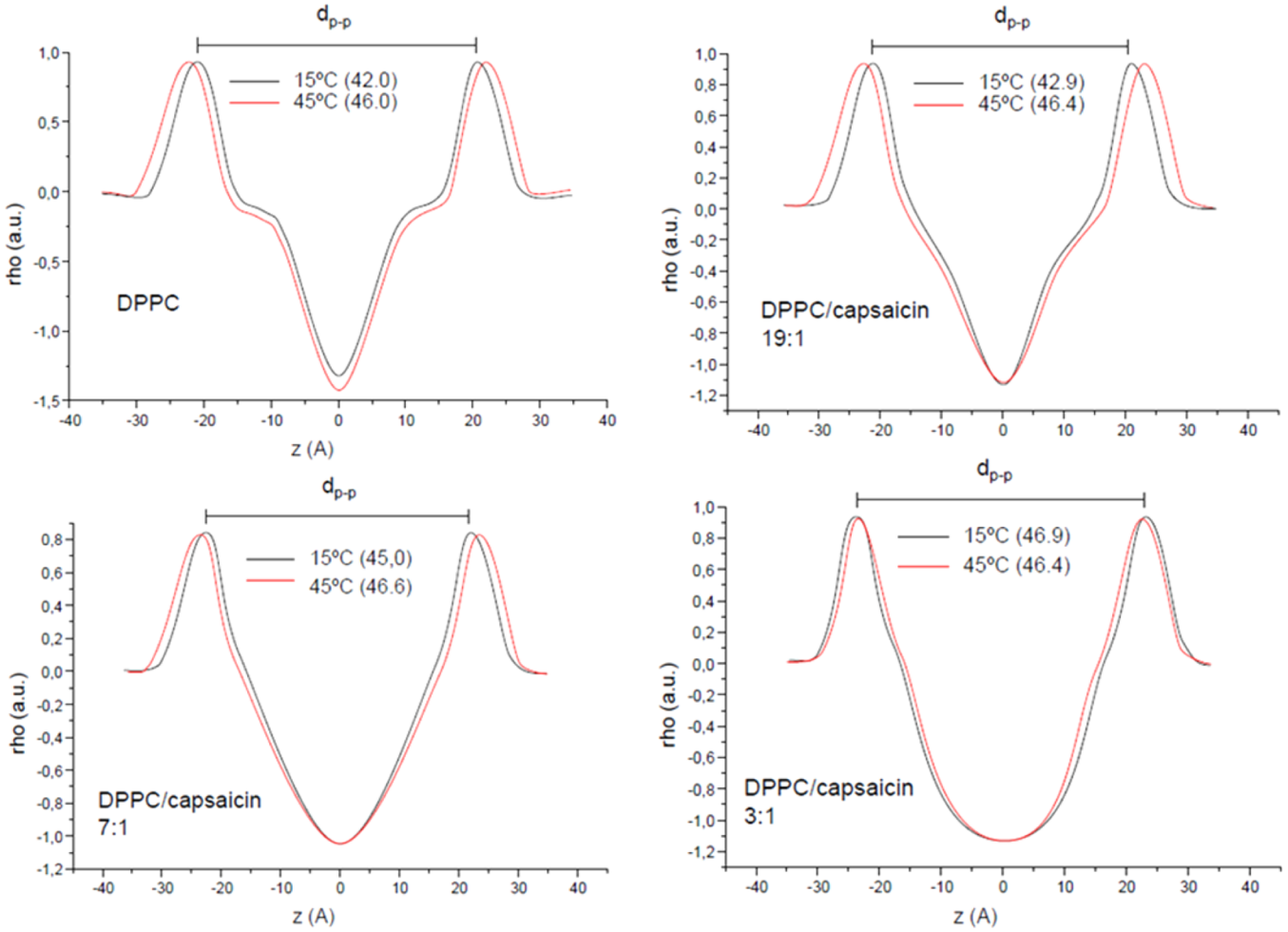

Figure 3. Small angle X-ray diffraction profiles at temperatures above (45 °C) and below (15 °C) DPPC/capsaicin mixture transition temperatures. The different compositions of the mixtures are given in DPPC/capsaicin molar ratio. Beside the temperatures, the distances between the phosphate groups of the bilayers (d_{p-p}) are given in Å (within parentheses).

Table 1. Chemical Shift Anisotropies, Δσ (ppm), of the ^{31}P-NMR Spectra Obtained from Pure DPPC and in the Presence of Different Concentrations of Capsaicin (DPPC/Capsaicin Molar Ratios), at Different Temperatures[a]

T (°C)	DPPC	19:1	7:1	3:1
26	68.1 ± 1.0	62.8 ± 0.3	51.9 ± 0.8	42.6 ± 0.5
30	63.1 ± 0.9	61.3 ± 0.2	46.6 ± 0.9	42.1 ± 0.7
34	60.4 ± 0.4	57.1 ± 1.2	42.7 ± 1.7	42.6 ± 0.9
38	47.6 ± 0.5	46.7 ± 1.6	42.6 ± 0.4	42.1 ± 0.4
52	45.5 ± 1.1	45.6 ± 0.9	42.3 ± 0.9	41.5 ± 0.4

[a]The figures shown are mean values ± SD from three measurements.

At temperatures below the transition temperature (T_c), the spectra were powder patterns characteristic of a gel phase. At 38 °C and higher, the spectra corresponded to a fluid-phase bilayer and were axially symmetric with some resolved quadrupole splittings arising from methylene segments in the acyl chains.

The spectra obtained after the addition of capsaicin to DPPC to give a DPPC/capsaicin molar ratio of 19:1 (Figure 4B) were very similar to those from pure phospholipid, except that the phase transition occurred at a lower temperature, and at 32 °C, the pattern of the spectrum already corresponded to a bilayer that is undergoing the transition from gel phase to fluid. A similar pattern was observed when the DPPC/capsaicin molar ratio was

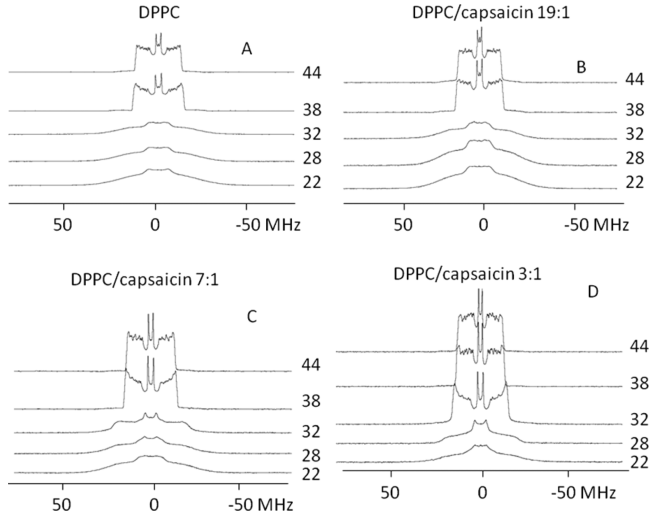

Figure 4. ^{2}H-spectra of mixtures composed by equimolar mixtures of DPPC-d_{62} and DPPC (mixtures named as DPPC) and capsaicin at the phospholipid/capsaicin molar ratios shown. The temperatures (in °C) are also given beside the spectra.

7:1 (Figure 4C), and in this case, the spectrum at 28 °C already indicated that the transition was taking place at this temperature.

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In the case of the 3:1 sample, Figure 4D revealed that the transition was also lowered and took place at 28 °C.

However, capsaicin had another interesting influence on the spectra. For example, the spectra corresponding to pure DPPC obtained at high temperatures exhibits a small deviation from a Pake line shape, indicating a slight elongation of the phospholipid vesicles in the external magnetic field used in these experiments.[23] These spectral changes affect the distribution of spectral intensity but not the effective deuterium quadrupole splitting. In the presence of capsaicin, a certain redistribution of spectral intensity close to maximum splitting was observed, while the methyl splittings remained unchanged, indicating that capsaicin reduced vesicle ellipticity.

In order to better discern the effect of capsaicin on membranes, the first spectral moment, M_1, was calculated for each spectrum. M_1 measures the average spectral width, and since each phase has a distinct spectral width, it will also have a characteristic M_1 that is proportional to the average order parameter. The variations in M_1 with temperature can be used to characterize membrane phase transitions and the molecular order of a membrane. Nevertheless, it should be taken into account that below the phase transition, the gel phase spectral shape is sensitive to dynamics. Pure DPPC (Figure 5A) showed values of about 140 kHz at low temperatures (below T_c), with a decrease to about 73 kHz at higher temperatures, clearly defining the phase transition, which started at about 35 °C and culminated at about 38 °C. The addition of capsaicin (Figure 5A) at 19:1 DPPC/capsaicin molar ratio did not changed M_1 values except at 36 °C, that is, within the range of coexistence of gel and fluid phases, with a considerably decrease from 117.7 kHz in DPPC to 83.8 kHz in the 19:1 sample. More dramatic effects were observed when the concentration of capsaicin was increased to give a DPPC/capsaicin 7:1 molar ratio, because in this case there was a clear decrease in the phase transition starting now at 28 °C and ending at about 34 °C. It is also noteworthy that a decrease in M_1 values was observed, especially for the 3:1 sample at all temperatures with respect to DPPC, indicating that capsaicin disorders the membrane hydrophobic core. This effect was clearer at temperatures below the phase transition.

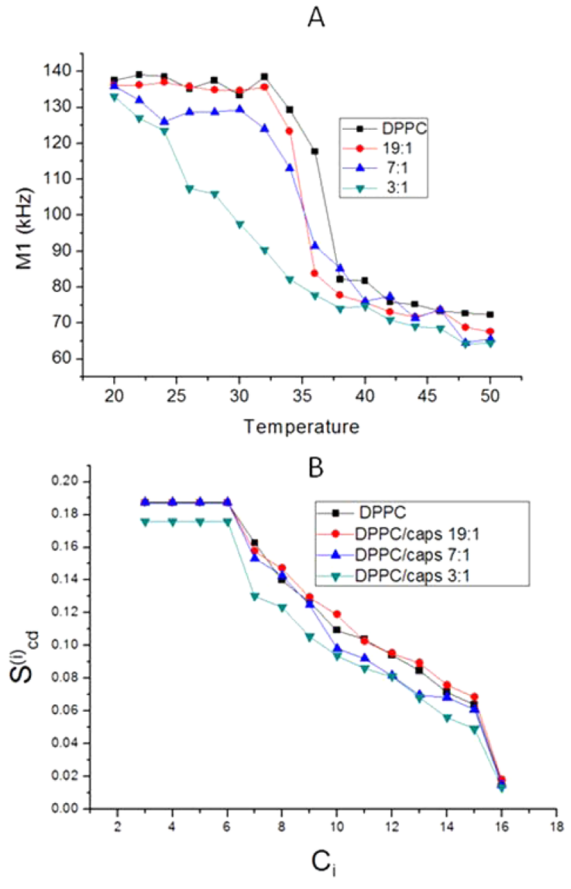

Figure 5. (A) Temperature-dependent changes of the M_1 and (B) smoothed order parameter profiles at 45 °C, for the DPPC/capsaicin mixtures as given beside the plots.

Since not all the individual methylene signals in the powder spectra shown in Figure 4 were completely resolved, the spectra above the transition temperature were transformed into oriented ones by means of the dePaking procedure (not shown). The quadrupole splittings ($\Delta\nu^i$), that is, the distances between the pairs of symmetric peaks, are related to the smoothed order parameters profiles S^i_{CD} of the various CD_2 acyl chain segments according to the equation

$$\Delta\nu^i = \frac{3}{4}\left(\frac{e^2Qq}{h}\right)S^i_{CD}$$

where e^2Qq/h is the quadrupole coupling constant, 167 kHz.[24] The smoothed order parameter is higher in the carbon segments next to the glycerol backbone and continuously decreases along the acyl chain toward the terminal methyl group. This gives rise to a peak pattern consisting of Pake doublets, with the outermost peak pair corresponding to the plateau of superimposed peaks from carbon segments 3–6[25,26] and the innermost pair of peaks representing the terminal methyls (not shown). As can be observed in Figure 5B, the smoothed order parameter profiles corresponding to 48 °C, S_{CD}, were not much changed for any carbon segment by the addition of capsaicin at a DPPC/capsaicin molar ratio of 19:1 with respect to pure DPPC, in agreement with the results observed when studying M_1 with respect to temperature (Figure 5A). However, clear decreases in the smoothed order parameter profiles were observed for higher concentrations of capsaicin, especially when the molar ratio was 3:1. At this ratio, the decrease affected all carbon segments, except the terminal methyl group. This suggests that the capsaicin molecule is set up in the membrane with its main axis parallel to the acyl chains of the phospholipid.

^{1}H NOESY MAS NMR Studies. This NMR technique was used to help with the localization of capsaicin in the membrane. The ^{1}H chemical shift assignments of POPC and capsaicin are shown in Figure 6A. POPC was used for these experiments because, at variance with DPPC, it forms fluid membranes at 25 °C and also allows the detection of specific ^{1}H-resonances thanks to its double bond, which correspond to the middle of the hydrophobic chains, whereas phospholipids like DPPC only allow the observation of a big resonance arising from most of the methylene protons of the fatty acyl chains. These assignments were confirmed by integration of peaks to estimate the number of protons from which each resonance was arising. Figure 6B shows the two-dimensional MAS-NOESY spectrum. Some important cross-peaks indicating intermolecular POPC−capsaicin interactions are highlighted. Interactions corresponding to the phenol ring and to the alkyl chain of capsaicin were observed. Only cross-peaks that were arising from nonoverlapping resonances are considered. In order to detect cross peaks arising from intramolecular cross relaxations, COSY experiments were carried

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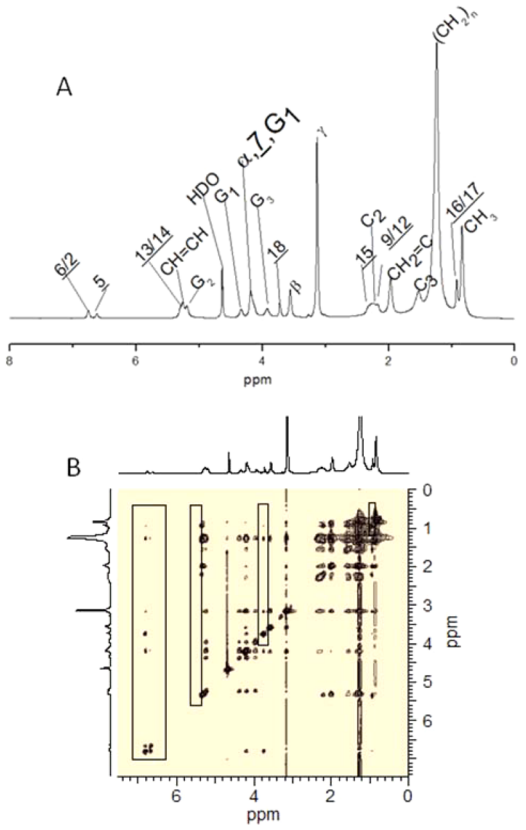

Figure 6. (A) ^{1}H MAS NMR spectrum of POPC/capsaicin (3:1 molar ratio). Assignments of the different resonances are done according to previous reports for POPC[46,47] and for capsaicin.[48] Assignments were confirmed by integration of the resonances and comparison between them. (B) ^{1}H NOESY MAS NMR of POPC/capsaicin (3:1 molar ratio), at a mixing time of 300 ms. Cross-peaks used to estimate interactions between phospholipid and capsaicin are highlighted.

out, and any peak also detected in this way was excluded from the analysis.

The cross-relaxation rate corresponds to the amount of magnetization transfer between capsaicin and POPC protons and allows the measurement of the frequency of collision between functional groups belonging to these molecules. In the single-mixing time approximation used in this work, cross-relaxation rates are proportional to normalized cross-peak volumes. These normalized volumes are plotted in Figure 7 for the different protons of capsaicin offering information allowing the estimation of the disposition of the capsaicin molecule in the membrane. It can be seen that protons bound to C18 of capsaicin, that is, belonging to the methoxy group bound to the phenol ring (Figure 7), interact mainly with protons bound to carbons G3 (glycerol backbone) and C3 of the fatty acyl chains of POPC and to a lesser extension with protons bound to carbons situated near the double bond of the oleoyl chain indicating that they are close to the beginning of the acyl chain. Moreover, the cross relaxation rate with $(CH_2)_n$ and CH_3 of the fatty acyl chains were very small, confirming that the phenolic ring of capsaicin is close to the membrane surface. In agreement with that, protons bound to carbons 2/6 and 5, all of them belonging to the phenol ring of capsaicin, are interacting mainly with protons bound to carbons of this region such as G3 and C3. On the other hand, protons bound to carbons 16/17 located at the end of the hydrophobic chain of capsaicin interact mainly with protons bound to carbons located at the center of the oleoyl chain as is the case of those around the double bond and also with C3. After these results, the disposition of the capsaicin molecule depicted in Figure 8 is proposed, showing that the phenol ring is located near the lipid−water interface, so that the hydroxyl group could interact with water. Given the interactions observed for the distal part of the nonenamidyl chain, it is proposed that the amide bond is also located near the lipid−water interface and the non-enamidyl chain is interacting with the segment of the fatty acyl chains of POPC from the ester bond to the center of the phospholipid monolayer, that is, to the proximity of the oleoyl double bond.

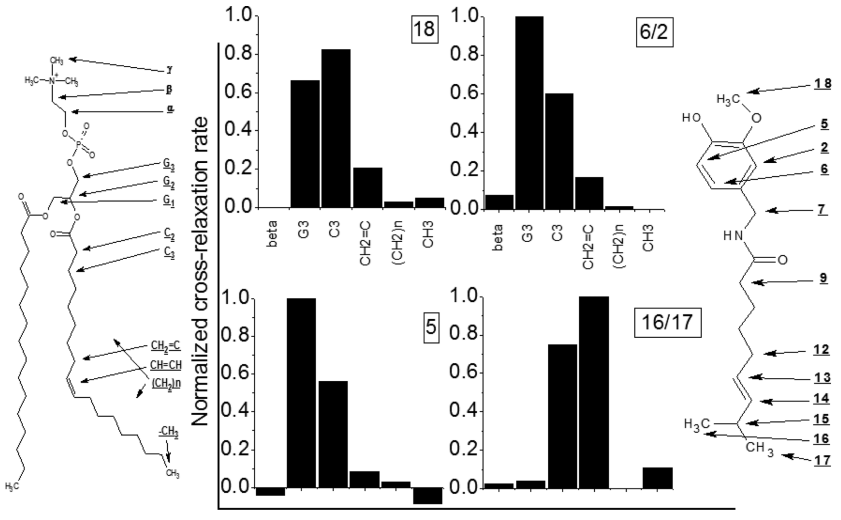

Figure 7. Normalized POPC−capsaicin cross-peaks obtained from the analysis of the ^{1}H NMR NOESY spectrum. Only cross-peaks that are not coming from overlapped resonances are used. Capsaicin proton assignments are shown at the right and POPC assignments at the left. POPC proton assignments are $N(CH3)_3$ (γ), $N-C\underline{H}_2-CH_2$ (β), $CH_2-C\underline{H}_2-O$ (α), $O-C\underline{H}_2-CH$ (G3), $CH_2-C\underline{H}-CH_2$ (G2), $CH-C\underline{H}_2-O$ (G1), $CH_2-C\underline{H}_2-COO$ (C2), $C\underline{H}_2-CH_2-COO$ (C3), $C\underline{H}_2{=}C$, $C\underline{H}{=}C\underline{H}$, $(C\underline{H}_2)$, and CH_3 (see references in the legend to Figure 6A).

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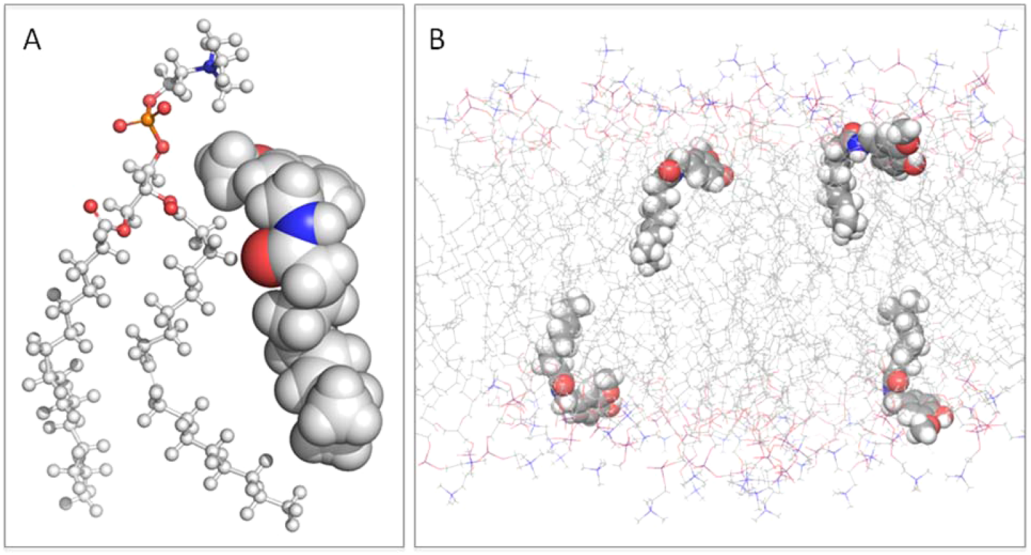

Figure 8. Proposed docking of POPC (POPC128a.pdb) and capsaicin deduced from the ^{1}H NOESY MAS NMR experiment. This diagram was elaborated by using PyMOL (www.pymol.org).

DISCUSSION

Given the wide variety of effects produced in cells by capsaicin and that its mode of action is not clear when acting on many proteins that are not specific receptors for this phytochemical, it is necessary to know in detail how this molecule interacts with biological structures. Since capsaicin possesses a hydrophobic character, it can be assumed that it will be preferentially localized in biomembranes, and certainly most of its biological effects can be traced to membrane proteins.[15] It was proposed that capsaicin affects the phase transition of DPPC, altering the structure of the membrane and creating fluid phase immiscibility and decreasing the lamellar L_{α} to H_{II} phase of DEPE, which is taken as an indication of alteration of the curvature of the membrane.[9] We have used in this paper DPPC and POPC membranes, because these are well characterized models very adequate for the use of the physical techniques employed here.

DSC shows alteration of the phase transition with formation of more than one structure, giving rise to capsaicin rich phases. The effect of capsaicin on DPPC membrane polymorphism is now documented in detail by SAXD and WAXD studies, showing that capsaicin insertion in DPPC membranes modifies its structure and increases the *d*-spacings in a concentration dependent manner that is more evident in the rigid gel state. Since the repetitive distance from which the *d*-spacing arises is the summation of the thickness of the lipid bilayer plus the associated water layer, the analysis of electronic density is necessary to discern which modifications are induced by capsaicin. This analysis proved that the increase in *d*-spacings observed with the membrane at both temperatures, below and above the gel to fluid transition, was mainly due to an increase in the thickness of water layer and only partially to the distances between both phosphate groups in the bilayer. It is noteworthy that the electronic profiles show that at high capsaicin concentrations such as 7:1 and 3:1 (DPPC/capsaicin molar ratios), there is a widening of the space located between both monolayers, indicating a condensation of the phospholipid layers. Since ^{31}P NMR did not detect any isotropic component (Supplementary Figure 2, in Supporting Information), the additional structures revealed by DSC should be DPPC/capsaicin complexes laterally segregated in the membrane.

The results obtained from ^{2}H NMR spectroscopy also show the fluidification effect produced by capsaicin in DPPC and the shift of the phase transition toward lower temperatures as the concentration of capsaicin was increased. The first spectral moment, M_1, shows that capsaicin decreases the order of the membrane at temperatures both above and below of the phase transition. The progressive decrease in the order of the bilayer induced by increasing concentrations of capsaicin was also confirmed by the S_{CD} smoothed order parameter profile obtained after the dePaking of the spectra, affecting most of the bilayer. It is noteworthy that ^{31}P NMR also revealed a fluidification effect of capsaicin, in this case focusing on the lipid−water interface. In order to localize capsaicin in the membrane, it should be noticed that $\Delta\sigma$ values indicate a remarkable fluidification in the polar headgroup even at 26 °C in the DPPC/capsaicin sample (3:1 molar ratio) (Table 1), whereas WAXD showed (Figure 2) that some order still existed at this temperature, and also ^{2}H NMR (Figure 5A) showed some order for this sample and temperature. It can be concluded that capsaicin affects more the order of the polar part of the membrane than that of the hydrophobic core, and this is in very good agreement with the ^{1}H-NOESY NMR experiments reported in this paper. Other previous studies have successfully used a combination of ^{31}P NMR and ^{2}H NMR to measure the peptide induced disorder of the membrane by antimicrobial peptides.[27−29]

In general, these results are indicative of the establishment of a molecular interaction between the phospholipid acyl chains and the capsaicin molecule, perturbing the cooperative behavior of the phospholipid, which can be explained by the intercalation of the capsaicin molecule between the DPPC molecules. Our ^{1}H NOESY MAS NMR studies confirm that part of the capsaicin molecule (the nine carbon alkyl chain) aligns itself principally with the main directions of the phospholipid acyl chains. According to our results, we propose a model (Figure 8) in which the hydroxyl and the amide groups of capsaicin are placed near the lipid/water interface allowing them to establish hydrogen bonding with water and other polar interactions with the polar head of the phospholipids. This location explains the perturbation of the phospholipid acyl chains, and the decrease of the onset of the gel to liquid-crystalline phase transition

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temperature observed by the incorporation of capsaicin to the membrane. This location of capsaicin allows the interaction of the molecule with the interfacial region of the bilayer and explains the previous observation indicating that the presence of capsaicin produces an increase in the fluorescence polarization of 2-anthroyloxystearic acid (2-AS), both below and above T_c.[9] Recent work on the TRPV1 channel has shown that capsaicin interacts with domains S1−S4 of this channel, and the tendency of this phytochemical to localize in the membrane as we describe will be fully compatible with this.[30,31] Additionally, molecular dynamics studies recently published supports the disposition in the membrane of the capsaicin molecule as it is deduced from our experimental data.[32]

It is widely documented in the literature that the alteration of membrane properties affects the function of proteins embedded in the membrane,[33] and this has been observed by membrane alterations induced by different phytochemicals.[15] In direct connection with that, the existence of a plasma membrane-dependent thermosensory pathway in mammalian cells has been demonstrated, whereby heat shock factor-1 (HSF-1) would become activated primarily through subtle changes in the fluidity state of the plasma membrane, which would in turn activate the transient opening of the specific TRPV1 channels, allowing a specific calcium-dependent signal to propagate and activate HSF-1.[34] The increase in fluidity produced by capsaicin has been found in several other biomembrane systems such as the gastrointestinal tracts,[8] bovine aortic endothelial cells, altering migration, which is an integral part of angiogenesis,[35] or HEK-293 cells in which capsaicin modulates the function of $GABA_A$ receptor by altering the membrane.[36]

High concentrations of capsaicin have been used in some experiments, especially in the case of ^{1}H NOESY MAS NMR, because this is the only way of visualizing capsaicin. .In other cases, like with ^{2}H NMR, ^{31}P NMR, DSC, and XRD a range of concentrations have been employed. In any case all techniques agree with respect the main conclusions of this work, as with the localization of capsaicin in the membrane and its fluidifying effect, at all the concentrations used.

In summary, we describe in detail the way in which capsaicin is located in the membrane and how it modifies the physical properties of the membrane with a loss of order, an increase in fluidity, and an increase in the thickness of the water layer in direct contact with the bilayer, explaining the many biological effects that have been described for this phytochemical.

MATERIALS AND METHODS

Materials. 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), and 1,2-dipalmitoyl-d_{62}-*sn*-glycero-3-phosphocholine (DPPC-d_{62}) were obtained from Avanti Polar Lipids (Birmingham, LA, U.S.A.). Capsaicin and all other chemicals were highly pure from Sigma Chemical Co. (Madrid, Spain).

Methods. *Preparation of Samples for DSC.* Samples were prepared by dissolving in chloroform/methanol (2:1) the desired amounts of lipids, including DPPC-d_{62} and nonlabeled DPPC in a 1:1 molar ratio. Capsaicin was also prepared in the same solvent and mixed with the phospholipid in the desired proportion. The mixture was then dried under a nitrogen stream and finally under vacuum. Samples were hydrated by dispersing them in 100 mM NaCl, 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), pH 7.4, buffer, using deuterium-depleted water, and multilamellar vesicles were prepared. The sample was then centrifuged, and the pellet was dispersed and resuspended at 55 °C, that is, at a temperature above the phase transition of DPPC, and 2 mg samples of phospholipid, pure or containing capsaicin, were resuspended in 0.5 mL of buffer and vortexed. The samples and the same volume of buffer (used as reference) were degassed for 10 min before loading the calorimeter. Thermograms were recorded by using a Microcal VP scanning calorimeter (Microcal, Northampton, MA). The scanning of all of the samples was carried out over a 10−90 °C temperature range at a heating rate of 60 °C/h. Data from the thermograms were recorded and analyzed using Microcal Origin 5.0 software. The traces were normalized, depending on the DPPC concentration of each sample. The thermal behavior of liposomes was evaluated by determining the linear onset and completion temperatures of the pretransition and the main transition (T_c) and by calculating ΔH corresponding to the main transition of DPPC in the samples.

X-ray Diffraction. Simultaneous small (SAXD) and wide (WAXD) angle X-ray diffraction measurements were carried out using a modified Kratky compact camera (MBraun-Graz-Optical Systems, Graz Austria), incorporating two coupled linear position sensitive detectors (PSD, MBraun, Garching, Germany) to monitor the *s*-ranges [$s = 2 \sin \theta/\lambda$, 2θ = scattering angle, $\lambda = 1.54$ Å] between 0.0075 and 0.07 and 0.20−0.29 Å^{-1}, respectively. Nickel-filtered Cu KR X-rays were generated by a Philips PW3830 X-ray generator operating at 50 kV and 30 mA. The detector position was calibrated by using silver stearate (small-angle region, *d*-spacing at 48.8 Å) and lupolen (wide angle region, *d*-spacing at 4.12 Å) as reference materials. Samples for X-ray diffraction were prepared as described above for DSC by mixing 15 mg of DPPC/DPPC-d_{62} in equimolar ratios and the appropriate amount of capsaicin in chloroform/methanol (2:1, molar ratio). Multilamellar vesicles were formed as described above for DSC measurements. After centrifugation at 13000*g*, the pellets were placed in a steel holder with cellophane windows, which provide a good thermal contact with the Peltier heating unit. X-ray diffraction profiles were obtained for 10 min exposure times after 10 min of temperature equilibration. Background corrected SAXD data were analyzed using the program GAP (global analysis program) written by Georg Pabst and obtained from the author.[37,38] This program allowed us to retrieve the membrane thickness, $d_B = 2(z_H + 2\sigma_H)$, from a full *q*-range analysis of the SAXD patterns.[39] The parameters z_H and σ_H are the position and width, respectively, of the Gaussian used to describe the electron-dense headgroup regions within the electron density model. The water layer was then given by $d_W = d - d_B$.

^{2}H NMR Measurements. Samples for ^{2}H NMR were prepared as described for DSC by using a total quantity of 15 mg of phospholipid, including perdeuterated and nonlabeled DPPC in a 1:1 molar ratio and capsaicin added to the desired proportion. Samples were hydrated by dispersing them in 100 mM NaCl, 25 mM Hepes, pH 7.4, buffer, using deuterium-depleted water, and multilamellar vesicles were prepared. The sample was then centrifuged, and the pellet was dispersed in 300 μL of buffer and transferred to NMR glass tubes.

^{2}H NMR experiments were carried out on a Bruker Avance 600 instrument (Bruker, Etlingen, Germany) at 92.123 MHz using the standard quadrupole echo sequence.[40] The spectral width was 150 kHz, with a 10 μs 90° pulse, 40 μs pulse spacing, 3.35 μs dwell time, 1 s recycling time, and 50 Hz line broadening, with an accumulation of 15000 transients. Spectra were acquired at temperatures ranging from 20 to 50 °C, raising the temperature in 2 °C steps. The first moment, M_1, was calculated for each spectrum of the different samples and at each temperature using the following equation:[41]

$$M_1 = \frac{1}{A} \sum_{\omega=-x}^{x} |\omega| f(\omega)$$

where ω is the frequency shift from the central (Larmor) frequency, $f(\omega)$ is the spectral intensity, x is the frequency shift range (between −60 and 60 kHz) and A is defined as

$$A = \sum_{\omega=-x}^{x} f(\omega)$$

Spectra were dePaked by numerical deconvolution with the software supplied by Bruker. The dePaked spectra correspond to the spectra that would be obtained from a planar membrane with its bilayer normal aligned parallel to the applied static magnetic field, thus enhancing

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resolution and facilitating analysis of individual spectral peaks.[25,42,43] These spectra were compared with the original ones to ensure that the relevant features were maintained during the dePaking process.

^{31}P NMR Measurements. The same samples used for ^{2}H NMR and the same spectrometer operating at 242.9 MHz were also used to collect ^{31}P NMR spectra. All spectra were obtained in the presence of a gated broad band proton decoupling (5 W input power during acquisition time), and accumulated free inductive decays were obtained from up to 8000 scans. A spectral width of 48536 Hz, a memory of 48536 data points, a 2 s interpulse time, and a 90° radio frequency pulse (11 μs) were used with inverse gated decoupling ^{1}H. Prior to Fourier transformation, an exponential multiplication was applied, resulting in a 100 Hz line broadening.

^{1}H NOESY MAS NMR. POPC (25 mg) and the appropriate amount of capsaicin were used to prepare multilamellar vesicles in deuterated water, by using a procedure similar to that described above for other NMR measurements. NMR experiments were performed on a Bruker Avance 600 spectrometer, operating at 600 MHz, equipped with a HRMAS probe and using ZrO_2 of 4 mm BL4 rotors with Kel-f BL4 caps at 25 °C. The spin rate was 8 kHz; data points were 1024 of 16 scans, and the spectral width was 20 ppm. The relaxation delay was 3.5 s, and the mixing time was 300 ms. Two-dimensional NOESY experiments were acquired using 90° pulses of 5.5 μs. Data were processed using TopSpin 2.1 software, supplied by Bruker.

The location probability was estimated from data obtained at a mixing time of 300 ms, according to[44]

$$\sigma_{ij} = \frac{(A_{ij}(t_m))}{(A_{jj}(t_m))Xt_m}$$

where σ_{ij} is the cross-relaxation rate, A_{ij} is the cross-peak volume, A_{jj} is the diagonal peak volume, and t_m is the mixing time of the NOESY spectrum. It has been discussed[17] that a single mixing time MAS-NOESY experiment is sufficient for characterizing intermolecular interactions in membranes as supported by previous extensive work.[44,45]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.5b00168.

DSC showing capsaicin effect on POPC and mixed POPC/POPG membranes and ^{31}P spectra of DPPC and DPPC/capsaicin membranes (PDF)

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Author Contributions

A.T. and A.M.F.M. carried out DSC and X-ray diffraction experiments; A.M.F.M., A.A., and A.M.deG. performed ^{2}H NMR and ^{31}P NMR experiments; M.S. and A.M.deG. did the ^{1}H NMR NOESY experiments; S.C.G. and J.C.G.F. planned the experiments; J.C.G.F. wrote most of the paper.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Caterina, M. J., Schumacher, M. A., Tominaga, M., Rosen, T. A., Levine, J. D., and Julius, D. (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature 389*, 816−824.

(2) Liao, M., Cao, E., Julius, D., and Cheng, Y. (2014) Single particle electron cryo-microscopy of a mammalian ion channel. *Curr. Opin. Struct. Biol. 27*, 1−7.

(3) 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.

(4) Hwang, Y. P., Yun, H. J., Choi, J. H., Han, E. H., Kim, H. G., Song, G. Y., Kwon, K. I., Jeong, T. C., and Jeong, H. G. (2011) Suppression of EGF-induced tumor cell migration and matrix metalloproteinase-9 expression by capsaicin via the inhibition of EGFR-mediated FAK/Akt, PKC/Raf/ERK, p38 MAPK, and AP-1 signaling. *Mol. Nutr. Food Res. 55*, 594−605.

(5) Zhu, F. X., Zhang, X. Y., Olszewski, M. A., and Robinson, N. E. (1997) Mechanism of capsaicin-induced relaxation in equine tracheal smooth muscle. *Am. J. Physiol. 273*, L997−1001.

(6) Hibino, Y., Morise, M., Ito, Y., Mizutani, T., Matsuno, T., Ito, S., Hashimoto, N., Sato, M., Kondo, M., Imaizumi, K., and Hasegawa, Y. (2011) Capsaicinoids regulate airway anion transporters through Rho kinase- and cyclic AMP-dependent mechanisms. *Am. J. Respir. Cell Mol. Biol. 45*, 684−691.

(7) Kalia, N. P., Mahajan, P., Mehra, R., Nargotra, A., Sharma, J. P., Koul, S., and Khan, I. A. (2012) Capsaicin, a novel inhibitor of the NorA efflux pump, reduces the intracellular invasion of Staphylococcus aureus. *J. Antimicrob. Chemother. 67*, 2401−2408.

(8) Meddings, J. B., Hogaboam, C. M., Tran, K., Reynolds, J. D., and Wallace, J. L. (1991) Capsaicin effects on non-neuronal plasma membranes. *Biochim. Biophys. Acta, Biomembr. 1070*, 43−50.

(9) Aranda, F. J., Villalain, J., and Gomezfernandez, J. C. (1995) CAPSAICIN AFFECTS THE STRUCTURE AND PHASE ORGANIZATION OF PHOSPHOLIPID-MEMBRANES. *Biochim. Biophys. Acta, Biomembr. 1234*, 225−234.

(10) Lundbaek, J. A., Birn, P., Tape, S. E., Toombes, G. E., Sogaard, R., Koeppe, R. E., 2nd, 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.

(11) Rizvi, S. I., and Luqman, S. (2003) Capsaicin-induced activation of erythrocyte membrane sodium/potassium and calcium adenosine triphosphatases. *Cell. Mol. Biol. Lett. 8*, 919−925.

(12) Perez-Lara, A., Ausili, A., Aranda, F. J., de Godos, A., Torrecillas, A., Corbalan-Garcia, S., and Gomez-Fernandez, J. C. (2010) Curcumin disorders 1,2-dipalmitoyl-sn-glycero-3-phosphocholine membranes and favors the formation of nonlamellar structures by 1,2-dielaidoyl-sn-glycero-3-phosphoethanolamine. *J. Phys. Chem. B 114*, 9778−9786.

(13) Barry, J., Fritz, M., Brender, J. R., Smith, P. E., Lee, D. K., and Ramamoorthy, A. (2009) Determining the effects of lipophilic drugs on membrane structure by solid-state NMR spectroscopy: the case of the antioxidant curcumin. *J. Am. Chem. Soc. 131*, 4490−4498.

(14) Ingolfsson, H. I., Koeppe, R. E., 2nd, and Andersen, O. S. (2007) Curcumin is a modulator of bilayer material properties. *Biochemistry 46*, 10384−10391.

(15) Ingolfsson, H. I., Thakur, P., Herold, K. F., Hobart, E. A., Ramsey, N. B., Periole, X., de Jong, D. H., Zwama, M., Yilmaz, D., Hall, K., Maretzky, T., Hemmings, H. C., Jr., Blobel, C., Marrink, S. J., Kocer, A., Sack, J. T., and Andersen, O. S. (2014) Phytochemicals perturb membranes and promiscuously alter protein function. *ACS Chem. Biol. 9*, 1788−1798.

DOI: 10.1021/acschemneuro.5b00168
ACS Chem. Neurosci. 2015, 6, 1741−1750

(16) Garcia-Garcia, J., Micol, V., de Godos, A., and Gomez-Fernandez, J. C. (1999) The cancer chemopreventive agent resveratrol is incorporated into model membranes and inhibits protein kinase C alpha activity. *Arch. Biochem. Biophys. 372*, 382−388.
(17) Siarheyeva, A., Lopez, J. J., and Glaubitz, C. (2006) Localization of multidrug transporter substrates within model membranes. *Biochemistry 45*, 6203−6211.
(18) Garcia-Garcia, J., Corbalan-Garcia, S., and Gomez-Fernandez, J. C. (1999) Effect of calcium and phosphatidic acid binding on the C2 domain of PKC alpha as studied by Fourier transform infrared spectroscopy. *Biochemistry 38*, 9667−9675.
(19) Perez-Lara, A., Corbalan-Garcia, S., and Gomez-Fernandez, J. C. (2011) Curcumin modulates PKC alpha activity by a membrane-dependent effect. *Arch. Biochem. Biophys. 513*, 36−41.
(20) Rappolt, M., Hickel, A., Bringezu, F., and Lohner, K. (2003) Mechanism of the lamellar/inverse hexagonal phase transition examined by high resolution x-ray diffraction. *Biophys. J. 84*, 3111−3122.
(21) Tardieu, A., Luzzati, V., and Reman, F. C. (1973) Structure and polymorphism of the hydrocarbon chains of lipids: a study of lecithin-water phases. *J. Mol. Biol. 75*, 711−733.
(22) Ruocco, M. J., and Shipley, G. G. (1982) Characterization of the sub-transition of hydrated dipalmitoylphosphtidylcholine bilayers. *Biochim. Biophys. Acta, Biomembr. 684*, 59−66.
(23) Brumm, T., Mops, A., Dolainsky, C., Bruckner, S., and Bayerl, T. M. (1992) Macroscopic orientation effects in broadline NMR-spectra of model membranes at high magnetic field strength: A method preventing such effects. *Biophys. J. 61*, 1018−1024.
(24) Burnett, L. J., and Muller, B. H. (1971) Deuteron Quadrupole Coupling Constants in Three Solid Deuterated Paraffin Hydrocarbons. *J. Chem. Phys. 55*, 5829−5831.
(25) Davis, J. H. (1979) Deuterium magnetic resonance study of the gel and liquid crystalline phases of dipalmitoyl phosphatidylcholine. *Biophys. J. 27*, 339−358.
(26) Seelig, J. (1977) Deuterium magnetic resonance: theory and application to lipid membranes. *Q. Rev. Biophys. 10*, 353−418.
(27) Lee, D. K., Bhunia, A., Kotler, S. A., and Ramamoorthy, A. (2015) Detergent-type membrane fragmentation by MSI-78, MSI-367, MSI-594, and MSI-843 antimicrobial peptides and inhibition by cholesterol: a solid-state nuclear magnetic resonance study. *Biochemistry 54*, 1897−1907.
(28) Lee, D. K., Wildman, K. H., and Ramamoorthy, A. (2004) Solid-state NMR spectroscopy of aligned lipid bilayers at low temperatures. *J. Am. Chem. Soc. 126*, 2318−2319.
(29) Henzler-Wildman, K. A., Martinez, G. V., Brown, M. F., and Ramamoorthy, A. (2004) Perturbation of the hydrophobic core of lipid bilayers by the human antimicrobial peptide LL-37. *Biochemistry 43*, 8459−8469.
(30) Cao, E., Liao, M., Cheng, Y., and Julius, D. (2013) TRPV1 structures in distinct conformations reveal activation mechanisms. *Nature 504*, 113−118.
(31) Liao, M., Cao, E., Julius, D., and Cheng, Y. (2013) Structure of the TRPV1 ion channel determined by electron cryo-microscopy. *Nature 504*, 107−112.
(32) Hanson, S. M., Newstead, S., Swartz, K. J., and Sansom, M. S. (2015) Capsaicin interaction with TRPV1 channels in a lipid bilayer: molecular dynamics simulation. *Biophys. J. 108*, 1425−1434.
(33) Gomez-Fernandez, J. C., Goni, F. M., Bach, D., Restall, C. J., and Chapman, D. (1980) Protein-lipid interaction. Biophysical studies of (Ca2+ + Mg2+)-ATPase reconstituted systems. *Biochim. Biophys. Acta, Biomembr. 598*, 502−516.
(34) Jeong, K. Y., and Seong, J. (2014) Neonatal capsaicin treatment in rats affects TRPV1-related noxious heat sensation and circadian body temperature rhythm. *J. Neurol. Sci. 341*, 58−63.
(35) Diplock, A. T., and Lucy, J. A. (1973) The biochemical modes of action of vitamin e and selenium: A hypothesis. *FEBS Lett. 29*, 205−210.
(36) Lainez, S., Valente, P., Ontoria-Oviedo, I., Estevez-Herrera, J., Camprubi-Robles, M., Ferrer-Montiel, A., and Planells-Cases, R. (2010) GABAA receptor associated protein (GABARAP) modulates TRPV1 expression and channel function and desensitization. *FASEB J. 24*, 1958−1970.
(37) Pabst, G., Rappolt, M., Amenitsch, H., and Laggner, P. (2000) Structural information from multilamellar liposomes at full hydration: full q-range fitting with high quality x-ray data. *Phys. Rev. E: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top. 62*, 4000−4009.
(38) Pabst, G., Koschuch, R., Pozo-Navas, B., Rappolt, M., Lohner, K., and Laggner, P. (2003) Structural analysis of weakly ordered membrane stacks. *J. Appl. Crystallogr. 36*, 1378−1388.
(39) Pabst, G. (2006) Global properties of biomimetic membranes: Perspectives on molecular features. *Biophys. Rev. Lett. 1*, 57−84.
(40) Davis, B. K., and Davis, N. V. (1983) Binding by glycoproteins of seminal plasma membrane vesicles accelerates decapacitation in rabbit spermatozoa. *Biochim. Biophys. Acta, Biomembr. 727*, 70−76.
(41) Hsueh, Y. W., Gilbert, K., Trandum, C., Zuckermann, M., and Thewalt, J. (2005) The effect of ergosterol on dipalmitoylphosphatidylcholine bilayers: a deuterium NMR and calorimetric study. *Biophys. J. 88*, 1799−1808.
(42) Davis, J. H. (1983) The description of membrane lipid conformation, order and dynamics by 2H-NMR. *Biochim. Biophys. Acta, Rev. Biomembr. 737*, 117−171.
(43) Sternin, E., Bloom, M., and MacKay, A. L. (1983) De-pake-ing of NMR spectra. *J. Magn. Reson. 55*, 274−282.
(44) Huster, D., Arnold, K., and Gawrisch, K. (1999) Investigation of Lipid Organization in Biological Membranes by Two-Dimensional Nuclear Overhauser Enhancement Spectroscopy. *J. Phys. Chem. B 103*, 243−251.
(45) Holte, L. L., and Gawrisch, K. (1997) Determining ethanol distribution in phospholipid multilayers with MAS-NOESY spectra. *Biochemistry 36*, 4669−4674.
(46) Scheidt, H. A., Pampel, A., Nissler, L., Gebhardt, R., and Huster, D. (2004) Investigation of the membrane localization and distribution of flavonoids by high-resolution magic angle spinning NMR spectroscopy. *Biochim. Biophys. Acta, Biomembr. 1663*, 97−107.
(47) Feller, S. E., Brown, C. A., Nizza, D. T., and Gawrisch, K. (2002) Nuclear Overhauser enhancement spectroscopy cross-relaxation rates and ethanol distribution across membranes. *Biophys. J. 82*, 1396−1404.
(48) Peng, A., Ye, H., Li, X., and Chen, L. (2009) Preparative separation of capsaicin and dihydrocapsaicin from Capsicum frutescens by high-speed counter-current chromatography. *J. Sep. Sci. 32*, 2967−2973.

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ACS Chem. Neurosci. 2015, 6, 1741−1750