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# Effect of curcumin on lateral diffusion in lipid bilayers

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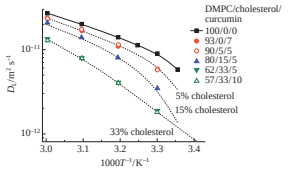
**Lateral diffusion in dimyristoylphosphatidylcholine lipid bilayers decreases in the presence of cholesterol and curcumin, as measured by $^1$H NMR spectroscopy, but the mechanisms of action of these two compounds are different.**




Curcumin is a natural yellow spice commonly used in Eastern kitchens and traditional medicines[1,2] due to its antioxidant, anti-carcinogenic, antimutagenic and antiinflammatory properties. It is believed that curcumin, like cholesterol, acts on a basic biological level such as biomembranes by changing the physical properties of a membrane rather than by directly binding to membrane proteins.[3] Cholesterol is an essential component of mammalian cell membranes, which can be embedded in the hydrophobic part of the membrane with its hydroxyl group interacting with the polar head groups of membrane phospholipids and sphingolipids.[4,5] Cholesterol increases membrane packing and ordering in a liquid crystalline phase.[6–8] Consequently, membrane fluidity gradually decreases with the concentration of cholesterol, as detected by measuring the lateral diffusion of phospholipids and sphingolipids.[9–11] In this work, we examined the combined effect of both curcumin and cholesterol on lipid lateral diffusion coefficient ($D_L$), reflecting a more biologically relevant condition compared to those of previous studies.

Figure 1 shows the structures of cholesterol, DMPC and curcumin. Glass-plate-oriented lipid multibilayers were prepared following a previously described procedure.[9,12] The amount of DMPC in each sample was 15 mg, while cholesterol and curcumin concentrations were varied to 33 and 10 mol%, respectively.† This range of concentrations was chosen because of a high solubility of cholesterol (~66 mol%[13]) and a relatively low solubility of curcumin (~10 mol%[14]). To prepare the test sample, a solution of DMPC in ethanol with an amount of cholesterol and curcumin was deposited (25 μl) on approximately 40 glass plates (5×14×0.08 mm). The solvent was evaporated in air and then in a vacuum overnight. The plates were stacked, placed in square, cross-sectioned tubes and hydrated in a humid atmosphere ($D_2O$) at 35 °C for five days. The degree of hydration of about 23 wt% was controlled by weighing the samples. The process of multi-bilayer formation was confirmed by the $^1$H NMR spectrum‡ of the sample oriented at a magic angle of 54.7°.[15] The final amount

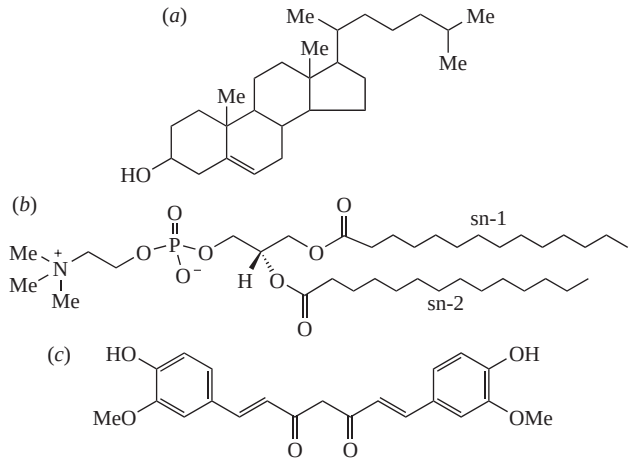



**Figure 1** Molecular structures of (*a*) cholesterol, (*b*) DMPC and (*c*) curcumin (keto form).

of water (45 wt%) was adjusted through a piece of filter paper placed on top of the glass stack. Afterward, the sample was sealed.

The NMR diffusion measurements in oriented lipid membranes were described in detail elsewhere.[9] An NMR goniometer probe was used to orient macroscopically aligned bilayers with the lipid bilayer normal at a magic angle (54.7°) with respect to the main magnetic field (Cryomagnet system).[15] For all measurements, a stimulated echo pulse sequence was used.[16] Diffusion decays $A(k)$ were obtained, where $A(k)$ is the spectrum integral, $k = \gamma^2\delta^2 g^2 t_d$, $\gamma$ is the $^1$H gyromagnetic ratio, $\delta$ is the duration, $g$ is the amplitude of the gradient pulse, $t_d = (\Delta - \delta/3)$ is the diffusion time, and $\Delta$ is the duration between identical gradient pulses.

The formation of plain-oriented multi-bilayers in all of the DMPC/cholesterol/curcumin test samples was confirmed by resolved $^1$H NMR spectra when the bilayers were oriented by their normal at the magic angle at temperatures higher than the main gel-to-liquid phase temperature of DMPC. As the magic

† Commercial DMPC from Avanti Polar Lipids and curcumin (>94% curcuminoid, >80% curcumin) and deuterated water (99.7% $D_2O$) from Sigma were used.

‡ A Chemagnetic InfinityPlus CMX-360 NMR spectrometer (Agilent) operating at a frequency of 360 MHz was used.

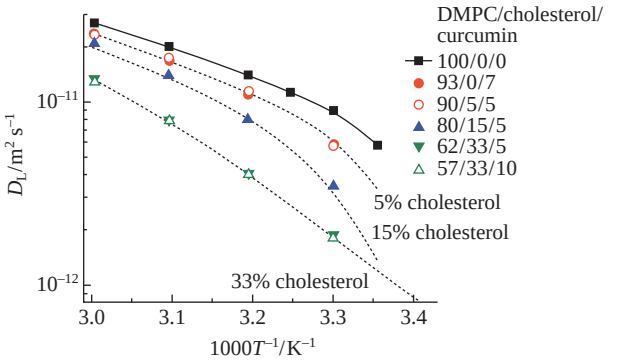



**Figure 2** Arrhenius plots of the lateral diffusion coefficients for DMPC/cholesterol/curcumin bilayers. Data for bilayers of DMPC (squares, solid line),[9,10,14] DMPC/cholesterol (dotted lines and legends),[9,10] DMPC/curcumin at 7 mol% of curcumin (red circles)[14] are shown for comparison. The measurement errors do not exceed the height of the symbols used.

angle of the sample orientation $\theta_{LD}$ deviates by more than 3° from 54.7°, the spin-echo and stimulated echo signals diminished to zero. Therefore, no signs of non-lamellar (hexagonal or cubic) phases were observed. Additionally, we demonstrated that the $^{31}$P NMR spectra have shapes typical of the lamellar phase of lipids.

Diffusion decays of the $^{1}$H NMR stimulated-echo for all bilayers of DMPC/cholesterol/curcumin were exponential, similar to the bilayers of DMPC, DMPC/cholesterol and DMPC/curcumin.[9,14,17] The slopes of semilogarithmic plots were independent of diffusion time, demonstrating a homogeneity of the translational mobility in the bilayers in the time-scale of these diffusion experiments ($t_d$ > 100 ms). These decays were analyzed, and the calculated values of $D_L$, alongside the lateral diffusion coefficients of DMPC, DMPC/cholesterol and DMPC/curcumin bilayers, are shown in Figure 2.

Figure 2 indicates that an increase in the cholesterol concentration from 0 to 33 mol% in DMPC bilayers leads to a monotonic decrease in the $D_L$ of DMPC across the entire range of concentrations.[9,17] This is related to the compressibility of the lipid bilayer. A decrease in the lateral diffusion coefficient of lipids in the presence of cholesterol has been described in terms of the molecular free-volumes:[18] cholesterol condenses a lipid membrane by decreasing free volumes of lipids, ordering lipid chains and decreasing the probability of molecular jumps between the equilibrium states of molecules in a membrane matrix. The presence of curcumin also diminishes the $D_L$ of DMPC at concentrations up to 10 mol% (Figure 2).[14] Similarities in the actions of curcumin and cholesterol on biomembranes have been suggested in biochemical studies.[19–21] It has been suggested that curcumin inserts deep into the membrane in a transbilayer orientation, anchored through the hydrogen bonding of the phenolic OH groups of curcumin to the phosphate group of the lipids and the hydrophobic interaction of the aromatic rings of curcumin with the phospholipid acyl chains in a manner analogous to cholesterol. Like cholesterol, curcumin binds DMPC in a transbilayer orientation at low concentrations and induces segmental ordering in the lipid membrane.[22] According to that idea, the addition of curcumin to the DMPC/cholesterol membrane should further compress the membrane and further decrease $D_L$, which is not in agreement with our experimental data. Another suggestion is that curcumin interacts mainly with a lipid interface (lipid–water interface) near the lipid headgroup (glycerol) region.[23–25] It was confirmed by an elimination of the pre-transition with increased curcumin concentration in DMPC and DPPC bilayers[21] and a much weaker overall effect of curcumin on $D_L$ and quadrupolar $^{2}$H NMR splitting of DMPC bilayers.[14] Indeed, the interaction of cholesterol with DMPC alkyl chains occurs in the whole extension of the chains; therefore, it is rather strong. Insertion of curcumin in lipid bilayers is a high energetic process in comparison with the adsorption of curcumin on the lipid surface, even in bilayers without cholesterol;[19,20] therefore, in more tightly packed DMPC/cholesterol membranes, it should take even more energy and become less probable.

The overwhelming effect of cholesterol over curcumin probably has biological significance. Indeed, if curcumin and other phenolic drugs only slightly modulate the biological membrane surface (or just the properties of the membrane surface), this may not disturb the structure and essential functions of living cells.

Curcumin at concentrations to 10 mol% slightly decreases the lateral diffusion of phospholipids in bilayers without cholesterol, but in the presence of 5–33 mol% cholesterol in dimyristoylphosphatidylcholine bilayers, the effect of curcumin is very small because in direct competition to insert into the DMPC lipid bilayer during bilayer formation from an initially homogeneous mixture, cholesterol overwhelmingly wins and consequently inhibits the insertion of curcumin.

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## References

1 B. Joe, M. Vijaykumar and B. R. Lokesh, *Crit. Rev. Food Sci.*, 2004, **44**, 97.
2 A. Goel, A. B. Kunnumakkara and B. B. Aggarwal, *Biochem. Pharmacol*., 2008, **75**, 787.
3 H. I. Ingolfsson, R. E. Koeppe and O. S. Andersen, *Biochemistry*, 2007, **46**, 10384.
4 A. M. Smondryev and M. Berkowitz, *Biophys. J.*, 1999, **77**, 2075.
5 M. Y. Pasenkiewicz-Gierula, T. Róg, K. Kitamura and A. Kusumi, *Biophys. J.*, 2000, **78**, 1376.
6 R. A. Haberkorn, R. G. Griffin, M. Meadows and E. Oldfield, *J. Am. Chem. Soc*., 1977, **99**, 7353.
7 E. Oldfield, M. Meadows, D. Rice and R. Jacobs, *Biochemistry*, 1978, **17**, 2727.
8 E. Falck, M. Parta, M. Karttunen, M. T. Hyvönen and I. Vattulainen, *J. Phys. Chem.*, 2004, **121**, 12676.
9 A. Filippov, G. Orädd and G. Lindblom, *Biophys. J.*, 2003, **84**, 3079.
10 A. Filippov, G. Orädd and G. Lindblom, *Langmuir*, 2003, **19**, 6397.
11 A. Filippov and O. N. Antzutkin, *Mendeleev Commun.*, 2013, **23**, 316.
12 A. V. Filippov, M. A. Rudakova and B. V. Munavirov, *Magn. Reson. Chem.*, 2010, **48**, 945.
13 J. Huang and G. W. Feigenson, *Biophys. J.*, 1999, **76**, 2142.
14 A. V. Filippov, S. A. Kotenkov, B. V. Munavirov and O. N. Antzutkin, *Langmuir*, 2014, **30**, 10686.
15 G. Orädd and G. Lindblom, *Magn. Reson. Chem*., 2004, **42**, 123.
16 J. E. Tanner, *J. Chem. Phys*., 1970, **52**, 2523.
17 G. Lindblom, G. Orädd and A. Filippov, *Chem. Phys. Lipids*, 2006, **141**, 179.
18 A. Filippov, G. Orädd and G. Lindblom, *Biophys. J*., 2007, **93**, 3182.
19 Y. Sun, C.-C. Lee, W. C. Hung, F.-Y. Chen, M.-T. Lee and H. W. Huang, *Biophys. J*., 2008, **95**, 2318.
20 W.-C. Hung, F.-Y. Chen, C.-C. Lee, Y. Sun, M.-T. Lee and H. W. Huang, *Biophys. J*., 2008, **94**, 4331.
21 J. Barry, M. Fritz, J. R. Brender, P. E. S. Smith, D.-K. Lee and A. Ramamoorthy, *J. Am. Chem. Soc*., 2009, **131**, 4490.
22 G.-F. Chen, Y.-Y. Chen, N.-N. Yang, X.-J. Zhu, L.-Z. Sun and G.-X. Li, *Sci. China Life Sci.*, 2012, **55**, 527.
23 H. I. Ingólfsson, P. Thakur, K. F. Herold, E. A. Hobart, N. B. Ramsey, X. Perole, D. H. de Jong, M. Zwata, D, Yilmaz, K. Hall, T. Maretzky, H. C. Hemmings, Jr., C. Blobel, S. J. Marrink, A. Koçer, J. T. Sack and O. S. Andersen, *ACS Chem. Biol.*, 2014, **9**, 1788.
24 M. Tsukamoto, K. Kuroda, A. Ramamoorthy and K. Yasuhara, *Chem. Commun*., 2014, **50**, 3427.
25 W. Kopec, J. Telenius and H. Khandelia, *FEBS J.*, 2013, **280**, 2785.

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