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Clustering of spin-labeled cholesterol analog diluted in bilayers of saturated and unsaturated phospholipids

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ABSTRACT

Clustering of spin-labeled cholesterol analog, 3β-doxyl-5α-cholestane (DChl), diluted in bilayers comprised of either saturated dipalmitoyl-glycero-phosphocholine (DPPC) or unsaturated dioleoyl-glycero-phosphocholine (DOPC) phospholipids was studied. DChl molar fraction X varied between 0.005 and 0.04. EPR spectroscopy applied at low temperatures (200 K) enabled exploring magnetic dipole-dipole (d-d) interaction between spin labels. For DOPC bilayers, EPR spectra were found to broaden remarkably with X increase. The broadening was simulated for the models of 2-dimentional (2-D) clusters with enhanced local concentration, X_{loc}, which was several times larger than X, and for 1-dimensional (1-D) DChl clusters. The distance of closest approach in these simulations attained the intermolecular lateral distance in the membrane (~0.7 nm). For DPPC bilayers, EPR spectra showed only small broadening, which in these simulations could not be reproduced even if X_{loc} was taken as small as X. However strong concentration dependence was found for electron spin echo (ESE) decays. Both the EPR and ESE data for DPPC bilayers were explained within the model assuming encapsulation of DChl molecules in lipid shells so preventing them to approach each other closer than a certain distance, $R_{\min}$. The $R_{\min}$ value was found to vary between ~2.5 nm and 5 nm, for X varying between 0.04 and 0.005; X_{loc} in these simulations was several times larger than X. So the DChl clustering in DOPC bilayers is driven by attractive lipid-mediated forces, while in DPPC bilayers long-range nanoscale lipid-mediated repulsive/attractive forces take place for distances smaller and larger $R_{\min}$, correspondingly.

1. Introduction

Cholesterol-lipid interactions and lateral structural organization of cholesterol-containing biological membranes are widely studied using different experimental and computational approaches – see e.g. [1–4]. It is known that cholesterol possesses the preferential affinity for saturated lipids which may be induced by the extended conformation adopted by saturated chains in the proximity of the steroid moiety. This different affinity is important for formation of nanoscale liquid-ordered assemblies – lipid rafts, – which are enriched with cholesterol and saturated chain lipids [1–12]. Lipid rafts are assumed to be involved in a variety of cell functions such as sorting, signaling, endocytosis, and protein activity [5–13]. The nature of rafts, their dynamics, physiological relevance, and other properties have not still been clearly understood. One may suggest that features of cholesterol-lipid and cholesterol-cholesterol interactions are important for elucidating the nature of raft formation.

Spin-label electron paramagnetic resonance (EPR) is widely used to study cholesterol-lipid interactions and lipid raft formation [14–18]. EPR allows to obtain phase diagrams of cholesterol-lipid mixtures and to get information on molecular dynamics and ordering. Pulsed EPR applied to solid-state systems is sensitive to magnetic dipole-dipole (d-d) interactions between spin labels, for their spatial separation at the nanoscale range [19–21], – i.e. for the range that is the most important for raft investigation. As it has been recently shown, under shock freezing of the membranes the lipid rafts may be captured [21]. The pulsed version of EPR – electron spin echo (ESE) technique – is highly sensitive to d-d measurements because inhomogeneous EPR line broadening is lifted in this technique [22].

It is known that spin-labeled cholesterol analog, 3β-doxyl-5α-cholestane (DChl), can be used to model dynamics and ordering of cholesterol in biological membranes [23–26]. In the present work, we

Abbreviations: EPR, electron paramagnetic resonance; ESE, electron spin echo; d-d interaction, magnetic dipole-dipolar interaction; DChl, 3β-doxyl-5α-cholestane; DOPC, dioleoyl-glycero-phosphocholine; DPPC, dipalmitoyl-glycero-phosphocholine; 1-D, one-dimensional; 2-D, two-dimensional

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study d-d interactions between DChl molecules by analyzing EPR and ESE data quantitatively. Previously, the EPR and ESE data for spin-labeled unsaturated dioleoyl-glycero-phosphocholine (DOPC) bilayers [19–21] have qualitatively shown the existence of lipid-mediated long-range intermolecular interactions, stretching up to several nanometers. Here we employ also saturated dipalmitoyl-glycero-phosphocholine (DPPC) bilayers – to explore the difference between unsaturated and saturated lipids for lipid-mediated long-range intermolecular interactions in the membranes. The DChl molar fraction, X, was varied between 0.005 and 0.04. Surprisingly, we found clustering of DChl molecules under these diluted conditions.

2. Experimental

The lipids DPPC and DOPC were obtained from Avanti Polar Lipids (Birmingham, AL, USA). DChl was obtained from Sigma-Aldrich (St. Louis, MO, USA). DPPC, DOPC or DPPC/DOPC mixture (1:1), taken with DChl of different molar fraction X, were co-dissolved in chloroform. The solvent was then removed by nitrogen flow, the samples were stored for 12 h under vacuum (10^{-2} bar) and then hydrated for 2 h at room temperature by adding the equal weight of bidistilled water. The samples with DPPC after hydration were heated up to 50 °C. These preparations resulted in formation of multi-lamellar vesicles. For low-temperature measurements, the samples were put in glass tubes of 3-mm outer diameter and frozen by immersion in liquid nitrogen. For measurements at room temperature, the samples were put in glass tubes of 1-mm outer diameter and studied immediately after preparation.

An ELEXSYS E580 9-GHz FT-EPR spectrometer (Bruker, Germany) was used, equipped with either a split-ring resonator (Bruker ER 4118X-MS3) or a dielectric resonator (Bruker ER 4118X-MD5) inside an Oxford Instruments CF 935 cryostat. The split-ring resonator was used in pulsed experiments, whereas the dielectric resonator was used in conventional stationary EPR experiments. The cryostat was cooled by flowing cold nitrogen gas, and the sample temperature was controlled with an accuracy of ± 0.5 K.

The incident microwave power in stationary EPR measurements was controlled to ensure the absence of saturation of EPR spectra. In pulse experiments, the resonator was over-coupled to provide a short ring time (~100 ns). A two-pulse ESE sequence was employed, with the pulse durations for the first and second pulses 16 and 32 ns, respectively, and the pulse amplitude adjusted to provide a maximum of the echo signal. The time delay τ between two pulses was scanned with a 4 ns step.

All calculations were performed with originally developed computer programs using the PascalABC.NET software.

3. Results and discussion

Low-temperature EPR spectra were recorded at temperature of 200 K for which motional effects in EPR spectra of nitroxide spin labels are suppressed (except for the small shift of the outer shoulders that is induced by fast librational motion [27–29]). Fig. 1 shows EPR spectra for DOPC, DPPC/DOPC (1:1) and DPPC bilayers with DChl admixture presenting at different molar fractions X. For convenience of comparison, all spectra are normalized by the amplitude of their low-field shoulder. One can see spectral broadening with X increasing for the cases of DOPC (Fig. 1a) and DPPC/DOPC (Fig. 1b) bilayers. The broadening is however very small for the case of DPPC bilayer (Fig. 1c).

The broadening with concentration increase at low temperature may be related only with the increase of d-d interactions between spin labels. We performed computer simulations of the influence of this interaction on EPR spectra, taking into account that spin labels in the bilayer may be assumed to be distributed in 2-dimensional (2-D) space [21]. Alternatively, a plane structure of the DChl molecule itself allows one to suggest lipid-mediated stacking interaction, resulting in 1-dimensional (1-D) DChl molecular arrangement [4,19,21]. The dipolar

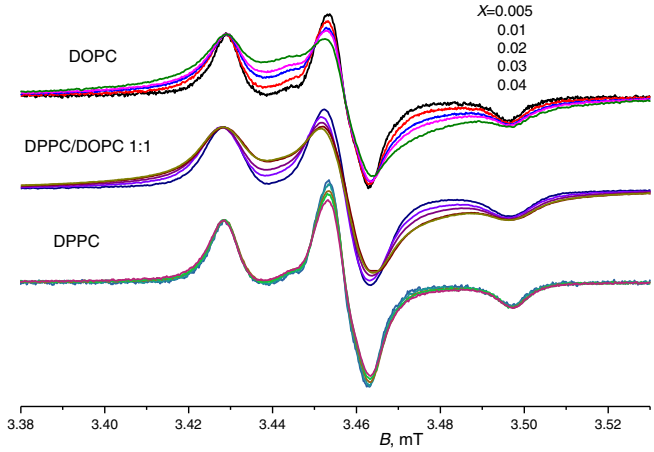

Fig. 1. EPR spectra taken at 200 K for DChl at different mole fraction X in the bilayers of indicated composition. Spectra are normalized to the same amplitude of the low-field shoulder; the larger X corresponds to the larger spectral distortions.

broadening was calculated for 2-D and 1-D spatial random distributions within a circle of the 100 nm radius in the former case and within a linear segment of the 200 nm length in the latter case. The result was convoluted with the EPR lineshape for the lowest DChl concentration employed in the experiment (X = 0.005). In the model, spin labels are assumed to be randomly distributed except of the distance of closest contact taken as 0.7 nm. (This value corresponds to the closest lipid-lipid distance in lipid bilayers [30–32]). The results of these calculations for the DOPC bilayer are shown in Fig. 2. One can see a qualitative agreement with the corresponding experimental data (cf. Fig. 1a) for both the 2-D (Fig. 2a) and 1-D (Fig. 2b) models.

Data in Fig. 2c show more precise quantitative comparison: here the best-fitted spectra for 2-D and 1-D models are compared with the experimental one, taken for X = 0.03. Note that only one fitting parameter was used in simulations: the local surface molar DChl

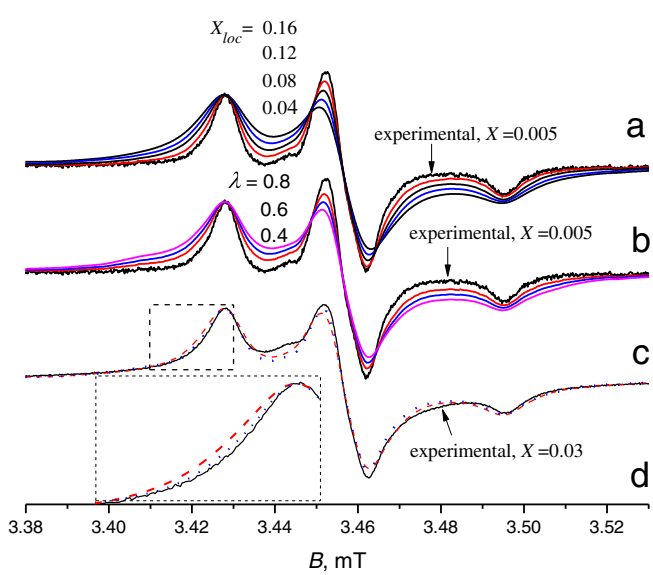

Fig. 2. (a) Experimental EPR spectra at 200 K for DChl in DOPC bilayer at X = 0.005 and its convolution with computed d-d broadening for the model of random distribution in 2-D clusters of different local concentration, X_{loc}. (b) The same for the case of 1-D cluster of different linear densities, λ, taken in nm^{-1} units. (c) The experimental spectrum for DChl in DOPC bilayer for X = 0.03 (solid line) and its fitting with the 2-D (dashed line, X_{loc} = 0.012) and 1-D (dotted line, λ = 0.6 nm^{-1}) cluster models. (d) The expansion of the data within the rectangle shown in (c).

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