Biochimica et Biophysica Acta 1808 (2011) 19–25

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

Transbilayer organization of membrane cholesterol at low concentrations: Implications in health and disease

Arunima Chaudhuri, Amitabha Chattopadhyay *

Centre for Cellular and Molecular Biology, Council of Scientific and Industrial Research, Uppal Road, Hyderabad 500 007, India

ARTICLE INFO

Article history:
Received 20 September 2010
Received in revised form 20 October 2010
Accepted 22 October 2010
Available online 28 October 2010

Keywords:
25-NBD-cholesterol
Cholesterol domain
Cholesterol transbilayer dimer
Dehydroergosterol
Membrane curvature
Membrane thickness

ABSTRACT

Cholesterol is an essential and representative lipid in higher eukaryotic cellular membranes and is often found distributed nonrandomly in domains in biological membranes. A large body of literature exists on the organization of cholesterol in plasma membranes or membranes with high cholesterol content. However, very little is known about organization of cholesterol in membranes containing low amounts of cholesterol such as the endoplasmic reticulum or inner mitochondrial membranes. In this review, we have traced the discovery and subsequent development of the concept of transbilayer cholesterol dimers (domains) in membranes at low concentrations. We have further discussed the role of membrane curvature and thickness on the transbilayer organization of cholesterol. Interestingly, this type of cholesterol organization could be relevant in cellular sorting and trafficking, and in pathological conditions.

© 2010 Elsevier B.V. All rights reserved.

Contents

1. Cholesterol: An essential and representative lipid in higher eukaryotes

Cholesterol is a major constituent in higher eukaryotic cellular membranes and is crucial in membrane organization, dynamics, function, and sorting [1–3]. Cholesterol is a predominantly hydrophobic molecule comprising a near planar tetracyclic fused steroid ring and a flexible isooctyl hydrocarbon tail (see Fig. 1a). The polar 3β-hydroxyl group provides cholesterol its amphiphilic character and helps cholesterol to orient and anchor in the membrane [4]. The tetracyclic nucleus and isooctyl side chain create the bulky wedge-type shape of the molecule. Interestingly, the planar tetracyclic ring system of cholesterol is asymmetric about the ring plane. The sterol ring has a flat and smooth side with no substituents (the α face) and a rough side with methyl substitutions (the β face; see Fig. 1b). The smooth α face of the sterol nucleus helps in favorable van der Waals interaction with the saturated fatty acyl chains of phospholipids [5]. The α face of cholesterol contains only axial hydrogen atoms. The absence of any bulky group in this face facilitates close contact between the sterol nucleus and phospholipid chains. The bumpiness

Abbreviations: 25-NBD-cholesterol, 25-[*N*-[(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-methyl]amino]-27-norcholesterol; DHE, dehydroergosterol ($\Delta^{5,7,9(11)22}$-ergostatetraen-3β-ol; DAPC, diarachidoyl-*sn*-glycero-3-phosphocholine; DLPC, dilauroyl-*sn*-glycero-3-phosphocholine); DMPC, dimyristoyl-*sn*-glycero-3-phosphocholine; DPPC, dipalmitoyl-*sn*-glycero-3-phosphocholine; DSPC, distearoyl-*sn*-glycero-3-phosphocholine; NBD, 7-nitrobenz-2-oxa-1,3-diazol-4-yl; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine

* Corresponding author. Tel.: +91 40 2719 2578; fax: +91 40 2716 0311.
E-mail address: amit@ccmb.res.in (A. Chattopadhyay).

0005-2736/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2010.10.013

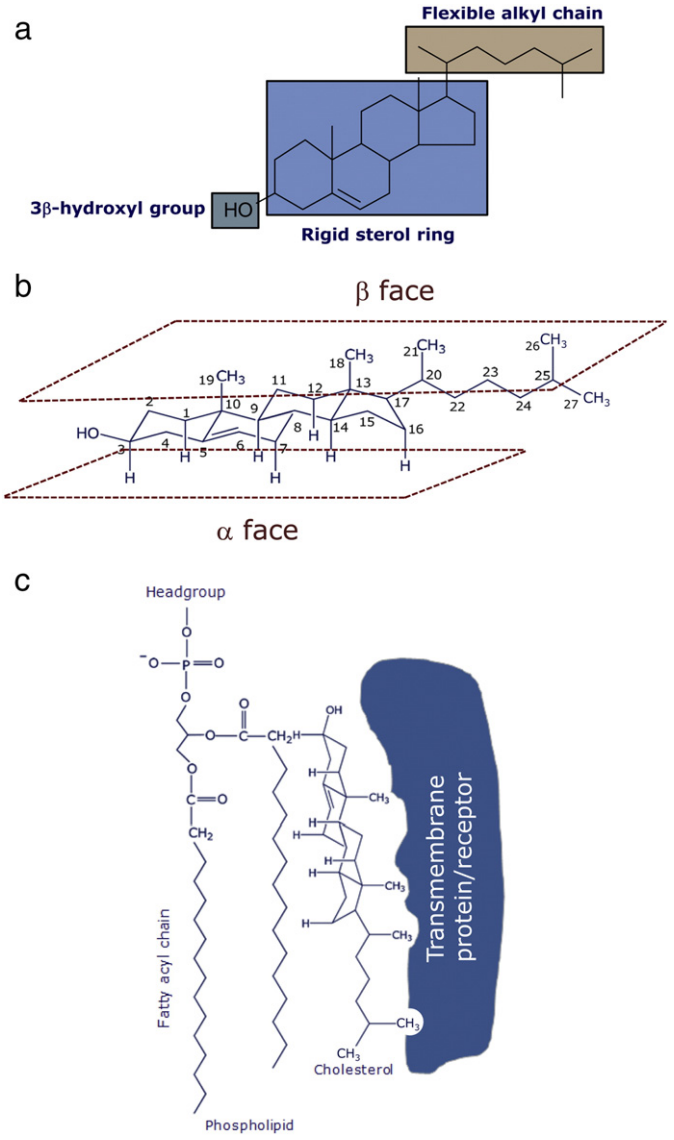

Fig. 1. Structure and orientation of cholesterol: (a) Chemical structure of cholesterol. Three structurally distinct regions are shown as shaded boxes: the 3β-hydroxyl group, the rigid steroid ring, and the flexible alkyl chain. The 3β-hydroxyl moiety is the only polar group in cholesterol thereby contributing to its amphiphilic character and it helps cholesterol to orient and anchor in the membrane. (b) Double faced cholesterol: cholesterol is characterized by a flat and smooth (α) face, and a rough (β) face. The α face of cholesterol contains only axial hydrogen atoms. The roughness of the β face is due to the protruding bulky methyl groups (see text for details). (c) A schematic diagram showing the orientation of cholesterol in relation to phospholipid molecules in membranes. The smooth α face of the sterol nucleus helps in favorable van der Waals interaction with the saturated fatty acyl chains of phospholipids. α and β faces of cholesterol can simultaneously interact with a saturated fatty acyl chain of phospholipids and uneven transmembrane domain of an integral membrane protein, respectively. Cholesterol is shown to align in bilayers with its 3β-hydroxyl group in the vicinity of the ester carbonyls of phospholipids and its tetracyclic ring immersed in the bilayer interior, in close contact with a part of the phospholipid fatty acyl chain. Adapted and modified from [29].

of the β face of cholesterol molecule is due to the protruding methyl groups at positions C18, C19 and the isooctyl chain linked to C17. The molecular structure of cholesterol is exceedingly fine-tuned over a very long time scale of natural evolution [6]. This is illustrated by recent reports that removal of methyl groups from cholesterol results in altered tilt angle which affects ordering and condensing effects, as shown by atomic scale molecular dynamics simulations [7,8]. Molecular simulation approaches have earlier shown that the α face of cholesterol promotes a stronger ordering effect on saturated alkyl chains compared to the β face [9]. In addition, it has been shown that cholesterol orients its smooth α face toward saturated chains and its uneven β face toward unsaturated chains of phospholipids [10], or with a bumpy transmembrane domain of an integral membrane protein (see Fig. 1c).

Cholesterol is oriented in the membrane bilayer with its long axis perpendicular to the plane of the membrane (Fig. 1c) so that its polar hydroxyl group encounters the aqueous environment and the hydrophobic steroid ring is oriented parallel to and immersed in the hydrophobic fatty acyl chains of the phospholipids [11]. It has been previously shown that cholesterol is aligned in bilayers with its 3β-hydroxyl group in the proximity of the ester bonds of phospholipids and its tetracyclic ring buried in the bilayer interior, in close contact with a part of the phospholipid fatty acyl chains [4,12]. It should be mentioned here that although the hydroxyl group of cholesterol is shown to be aligned at the level of *sn*-2 ester carbonyl group of the phospholipid in Fig. 1c, unambiguous experimental evidence supporting the interaction (hydrogen bonding) between the hydroxyl group of cholesterol and the lipid carbonyl group is lacking. Since the length of the cholesterol molecule including the isooctyl tail in *all-trans* energy minimum conformation is ~20 Å, a single cholesterol molecule can traverse one leaflet of a bilayer composed of phospholipids ([12]; see Fig. 1c).

2. Cholesterol domains in membranes

Cholesterol is often found distributed nonrandomly in domains in biological and model membranes [1,2,13–16]. Many of these domains (sometimes termed as 'lipid rafts') are believed to be important for the maintenance of membrane structure and function. A unique property of cholesterol which contributes to its capacity to form membrane domains is its ability to form liquid-ordered-like phase in higher eukaryotic plasma membranes [17]. The idea of such specialized membrane domains assumes significance in cell biology since physiologically important functions such as membrane sorting and trafficking [18], signal transduction processes [19], and the entry of pathogens [20–22] have been attributed to these domains. Importantly, cholesterol is distributed heterogeneously among various intracellular membranes. The lowest cholesterol concentration is found in the membranes of the endoplasmic reticulum, which interestingly is the site of cholesterol biosynthesis [23]. Cholesterol concentration increases progressively along the *cis*, *medial* and *trans* Golgi stacks [24,25], while the highest concentration (~90% of the total cellular cholesterol) is found in the plasma membrane [26]. In addition, cholesterol plays a vital role in the function and organization of membrane proteins and receptors [27–29].

3. Cholesterol domains in membranes at low concentrations

3.1. Early evidence of transbilayer cholesterol dimers from differential scanning calorimetric studies

A large body of literature exists on the organization of cholesterol in plasma membranes or membranes with high cholesterol content. Unfortunately, very little is known about its organization in the membrane when cholesterol content is very low (<5 mol%), similar to what is found in endoplasmic reticulum (where cholesterol biosynthesis takes place) and mitochondrial membranes [30]. It was initially assumed that cholesterol is distributed uniformly in the membrane at these concentrations. However, the evidence for this is quite tenuous even in earlier literature [31–33].

Evidence for specific organization of cholesterol molecules in membranes at low concentrations was obtained from three independent studies carried out by various groups [34–37]. Harris et al. [34] monitored the cryoscopic depression of multilamellar vesicles of DPPC/cholesterol (0–6 mol% cholesterol) and explained their results

on the basis of transbilayer dimer formation in the fluid phase at low concentrations. Differential scanning calorimetric studies showed that there was a sharpening of the phase transition (due to increase in the size of cooperative unit) by cholesterol at very low concentrations (0–5 mol%), instead of the broadening expected if chain melting was accompanied by continuously changing compositions of the two phases [34]. Further analysis of cholesterol–DPPC interaction, monitored by lowering of transition temperature as a function of increasing cholesterol concentration in DPPC membranes showed that the slope of the curve (of transition temperature vs. cholesterol concentration) was consistent with the molecular weight of a dimer when cholesterol concentration was 3 mol% and above. These results showed that while cholesterol is immiscible in the gel phase membrane and exists as separate solid domains, it forms monodisperse solutions below 2 mol% in the fluid phase. Based on space-filling considerations and control experiments with sterols lacking the isooctyl chain, these authors proposed a transbilayer dimer arrangement (see later). In this model, cholesterol molecules are localized rather deep in the membrane. This is consistent with the previously proposed deep location of cholesterol molecules across the hydrophobic core of the two leaflets of the membrane bilayer [38,39]. These dimers are stabilized by van der Waals interaction. Interestingly, no evidence for cholesterol dimerization was observed in lipid monolayers where transbilayer dimers cannot be formed [40].

3.2. Evidence of transbilayer dimers from fluorescent sterols

A major reason for the apparent paucity of reports describing organization of cholesterol in membranes at low concentrations is the lack of suitable techniques to monitor such structures. In this context, use of fluorescent sterols represents a powerful approach for studying cholesterol organization in membranes due to their high sensitivity, time resolution, and multiplicity of measurable parameters [41,42]. One class of probes commonly used for such studies is sterols that are covalently linked with extrinsic fluorophores. The advantage of this class of probes is the choice of the fluorescent label to be used, and therefore, specific probes with appropriate photophysical properties can be designed for specific applications. A widely used extrinsic fluorophore in biophysical, biochemical, and cell biological studies is the NBD group [43]. The NBD moiety possesses some of the most desirable properties for serving as an excellent probe for both spectroscopic [35,44–47] and microscopic [48,49] applications. It is very weakly fluorescent in water and upon transfer to hydrophobic media, it fluoresces brightly in the visible range and shows a large degree of environmental sensitivity [50–53]. The derivative of cholesterol, 25-NBD-cholesterol, in which the NBD group is attached to the flexible alkyl chain of cholesterol (see Fig. 2a), represents a useful fluorescent analogue of cholesterol. The detailed characterization of the spectroscopic properties of 25-NBD-cholesterol in model membranes and membrane-mimetic systems has been previously carried out [35,37,50,54–57]. The NBD group in this analogue has been found to be localized deep in the hydrocarbon region of the membrane, approximately 5–6 Å from the center of the bilayer, thereby indicating that the orientation of this analogue in the membrane is similar to that of cholesterol [50,54]. It has also previously been shown by electrophoretic measurements that 25-NBD-cholesterol is not charged when bound to membranes at neutral pH [50]. Interestingly, unlike phospholipids labeled in their acyl chains, the NBD group in 25-NBD-cholesterol has been shown not to loop back to the membrane interface possibly due to the stereochemical rigidity of the sterol ring and/or the reduction of hydrophilicity due to the methyl group (not present in NBD-labeled phospholipids) attached to the NBD moiety [43]. Interestingly, this unique orientation of 25-NBD-cholesterol offers a convenient means to localize a polar and potentially reactive group deep inside the membrane. The unique position of this probe in the membrane has

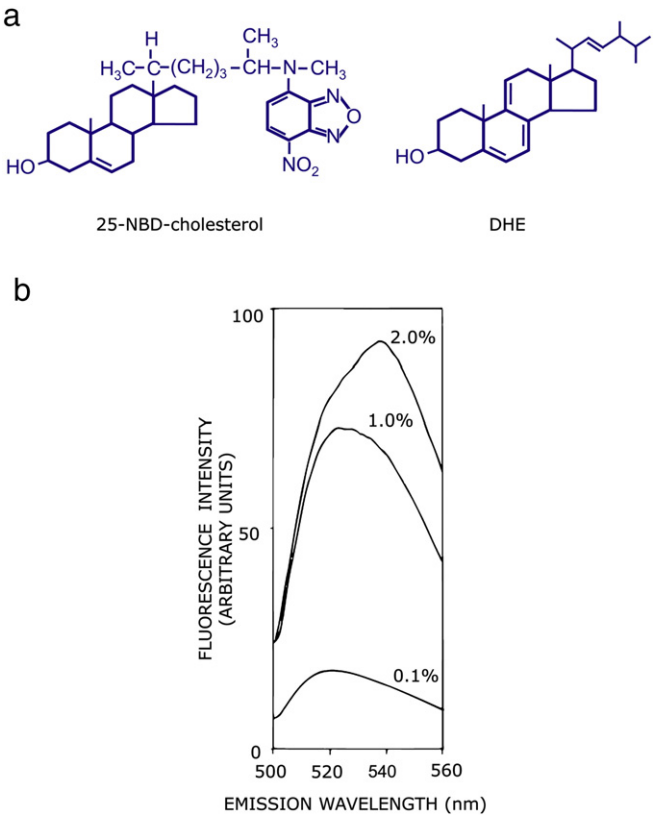

Fig. 2. (a) Chemical structures of fluorescent sterols. (b) Spectral signature reveals the presence of cholesterol dimers in membranes. Representative fluorescent emission spectral feature of 25-NBD-cholesterol in gel (ordered) phase DPPC membranes. The emission spectrum appears smooth and homogeneous when the concentration of 25-NBD-cholesterol is very low (0.1 mol%). The emission maximum is ~522 nm under this condition. This spectral feature represents monomers of cholesterol. With increase in cholesterol content, spectrum becomes broad and inhomogeneous accompanied by a shift in the emission maximum. A new peak is observed at ~539 nm when the concentration of 25-NBD-cholesterol reaches ~2 mol%. This signifies the presence of cholesterol dimers (see Fig. 3a). The dimer peak is observed at a higher concentration of cholesterol (~5 mol%) in fluid (liquid-disordered) phase. This characteristic spectral feature was attributed to specific non-covalent dimer of cholesterol and not due to non-specific aggregation of the NBD group (see [35,37] for details). The same conclusion was arrived by using the intrinsic fluorescence of DHE [36,37]. Adapted and modified from [35].

previously been exploited in fluorescence resonance energy transfer experiments to study the spatial orientation of specific sites on the chloroplast coupling factor in the membrane in reconstituted vesicles thereby providing further support for its orientation [55]. In contrast to sterols that are chemically linked to extrinsic fluorophores (such as 25-NBD-cholesterol), DHE represents a naturally occurring fluorescent cholesterol analogue that closely mimics cholesterol (see Fig. 2a for chemical structures). For this reason, DHE is extensively used in cellular studies involving cholesterol [58]. The issue of specific organization of cholesterol molecules in membranes at low concentrations has been amply explored by the use of fluorescent sterols, namely, 25-NBD-cholesterol [35,37,48,57] and dehydroergosterol [36,37].

In an elegant study, Mukherjee and Chattopadhyay [35] utilized aggregation-sensitive fluorescence characteristics of 25-NBD-cholesterol fluorescence to explore local organization of cholesterol in the membrane at very low concentrations. By careful analysis of the emission spectral features of 25-NBD-cholesterol in DPPC membranes at the concentration range of 0.1–5 mol%, the possible presence of transbilayer tail-to-tail dimers of cholesterol in such membranes was detected both in gel and fluid phases (see Figs. 2b and 3a for further details). Monitoring corresponding changes in the absorption spectrum, it was further shown, that the cholesterol dimers represented

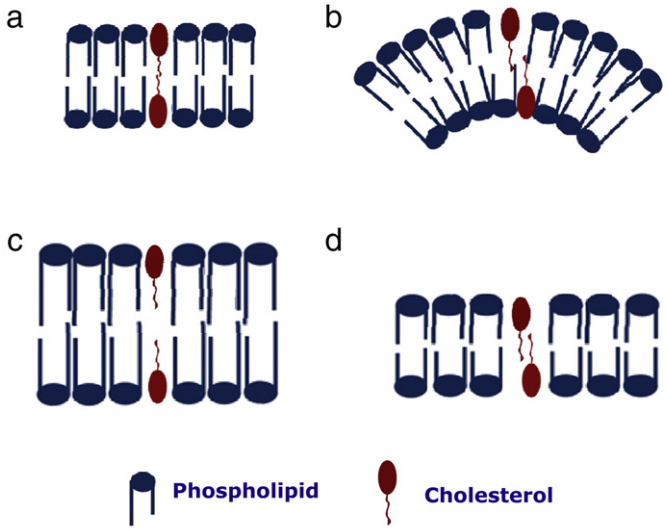

Fig. 3. A schematic diagram of the membrane bilayer showing the effects of curvature stress and membrane thickness on the organization of cholesterol dimers: (a) the transbilayer tail-to-tail dimers of cholesterol, (b) cholesterol monomers in membranes with high surface curvature such as small unilamellar vesicles, (c) cholesterol monomers in relatively thick membranes formed by phospholipids having longer fatty acyl chains, and (d) cholesterol monomers in relatively thin membranes formed by phospholipids having shorter fatty acyl chains. See text for details. Adapted and modified from [37].

the formation of a ground state complex (rather than an excited state interaction). By designing careful control experiments, the possibility that the unique spectral feature was due to non-specific aggregation of the NBD group was ruled out. In other words, these results provide novel information about cholesterol dimerization in membranes at low concentrations, rather than providing information on NBD–NBD interactions.

These results were further validated by Loura and Prieto [36] by utilizing the intrinsic fluorescence of DHE, a naturally occurring fluorescent cholesterol analogue found in yeast. DHE differs from cholesterol in having three additional double bonds and a methyl group (see Fig. 2a). A number of studies have shown that DHE faithfully mimics cholesterol in biophysical, biochemical and cell biological studies [13,58]. Loura and Prieto [36] analyzed DHE concentration-dependent depolarization in membranes using a theoretical framework and explained their results on the basis of transbilayer tail-to-tail dimers of cholesterol, previously reported by us [35] and others [34]. These conclusions were further supported by measurements in which the change in peak intensity ratio of the vibronic bands of DHE was monitored as a function of DHE concentration [37]. In addition, Loura and Prieto [36] also indicated that cholesterol dimers could be sensitive to membrane curvature.

3.3. Effects of curvature stress and membrane thickness

The effect of membrane curvature stress on the transbilayer organization of cholesterol in membranes was comprehensively addressed by Rukmini et al. [37]. By monitoring the distinct spectral feature of 25-NBD-cholesterol in membranes of varying curvature, it was shown that the transbilayer dimer arrangement is sensitive to membrane curvature and dimerization is not favored in highly curved membranes (such as small unilamellar vesicles; see Fig. 3b). This was attributed to differential packing arrangements in the two leaflets of highly curved, small vesicles. Interestingly, cholesterol dimerization was favored upon release of curvature stress by reforming larger vesicles (with less curvature stress) from small vesicles by extrusion, as evidenced from reversible modulation of spectral features [37].

A major issue that required to be validated with respect to cholesterol dimers is their transverse (as opposed to lateral) nature. This is relevant since the spectral features used for the above studies are not always capable of distinguishing transverse dimers from lateral dimers. In addition, lateral dimers of cholesterol have been previously reported in organic solvents [59]. This issue was resolved by incorporating 25-NBD-cholesterol in membrane bilayers of varying thickness (by using saturated phosphatidylcholines containing fatty acyl chains of 12–20 carbon atoms). Interestingly, the characteristic spectral feature for cholesterol dimers was found only in membranes formed from DPPC and DMPC but not in bilayers formed by DAPC, DLPC and DSPC [37]. Taken together, these results showed that the process of transbilayer dimerization is stringently controlled by a narrow window of membrane thickness (see Fig. 3c and d). These results assume significance in the context of increasing membrane thickness from Golgi to the plasma membrane due to the gradient in cholesterol concentration [23].

3.4. Organization and dynamics of transbilayer dimers

The organization of the transbilayer dimers merits comment. The microenvironmental features of cholesterol monomers and dimers were explored by Mukherjee and Chattopadhyay [57] utilizing wavelength-selective fluorescence spectroscopy. Wavelength-selective fluorescence comprises a set of approaches based on the red edge effect in fluorescence spectroscopy that can be used to directly monitor the environment and dynamics around an organized molecular assembly [60–62]. A shift in the wavelength of maximum fluorescence emission toward higher wavelengths, caused by a shift in the excitation wavelength toward the red edge of absorption band, is termed red edge excitation shift (REES). This effect is mostly observed with polar fluorophores in motionally restricted media such as very viscous solutions or condensed phases where the dipolar relaxation time for the solvent shell around a fluorophore is comparable to or longer than its fluorescence lifetime [60–62]. REES arises due to slow rates of solvent relaxation (reorientation) around an excited state fluorophore, which depends on the motional restriction imposed on the solvent molecules (or the dipolar environment, as in green fluorescent protein [63]) in the immediate vicinity of the fluorophore. The unique feature of REES is that while other fluorescence techniques yield information about the fluorophore itself, REES provides information about the relative rates of solvent relaxation that is not possible to obtain by other techniques. It has been previously shown that REES serves as a sensitive tool to monitor the organization and dynamics of organized molecular assemblies such as membranes and membrane-mimetic systems [44–46,64].

Mukherjee and Chattopadhyay [57] monitored the microenvironment of cholesterol dimers utilizing REES of the NBD group in 25-NBD-cholesterol. By analysis of REES results, obtained in conditions favoring predominantly either monomers or dimers of cholesterol, it was shown that the microenvironment around the cholesterol dimer offers more restriction to solvent reorientation in the excited state and therefore appears to be more rigid. The relatively rigid microenvironmental features of cholesterol dimers possibly slow down the rate of solvent reorientation giving rise to REES effects. Interestingly, monomers of 25-NBD-cholesterol do not display any REES [57]. These results show that REES is sensitive to the organization of cholesterol in the membrane, when probed using 25-NBD-cholesterol. These results were further supported by corresponding changes in fluorescence anisotropy as a function of excitation wavelength.

The lateral dynamics (diffusion) of cholesterol dimers assumes significance in the context of relatively rigid microenvironment of the dimer. Pucadyil et al. [48] utilized a novel version of fluorescence recovery after photobleaching (FRAP) measurements to provide estimates for lateral diffusion coefficients of cholesterol dimers and monomers using 25-NBD-cholesterol. In these measurements,

'wavelength-selective FRAP' measurements were carried out in DPPC membranes containing 25-NBD-cholesterol. The diffusion properties of the transbilayer dimer (evident from spectral features) and monomer were derived by analysis of FRAP results after photoselecting a given population by use of specific wavelength-range characteristic of that population. Based on this approach, the results showed that the organization of 25-NBD-cholesterol in DPPC membranes is heterogeneous, with the presence of fast- and slow-diffusing species. The presence of fast- and slow-diffusing populations of 25-NBD-cholesterol was interpreted to correspond to predominant populations of cholesterol monomers and dimers. Interestingly, the estimated molecular weights of the fast- and slow-diffusing species exhibited excellent agreement with monomer and dimer of 25-NBD-cholesterol. This reinforces that the fast- and slow-diffusing populations correspond to cholesterol monomers and dimers.

4. Transbilayer dimers of cholesterol: Implications in cellular sorting and disease

Since there are a number of intracellular organelles such as the endoplasmic reticulum (ER) and the mitochondrial membrane where the cholesterol content is actively maintained at a low level [30], any information on cholesterol organization in such membranes will be useful. Cholesterol domains found in membranes of low cholesterol content (such as the ER or the inner mitochondrial membrane) could have important functional implications in intracellular sorting and trafficking. The low cholesterol content of the ER membrane has been attributed to its need to be readily deformable for newly synthesized membrane proteins to be folded and assembled properly [23]. It has been previously reported that low amounts (<3 mol%) of cholesterol contribute to softness of the membrane [65,66]. These authors found that cholesterol in amounts up to 3 mol% softens the lipid bilayer and only at concentrations higher than 4 mol% leads to the well known effect of rigidification which is responsible for the mechanical coherence of eukaryotic plasma membranes. It has been suggested that the specific gradient of cholesterol concentration found in Golgi and ER facilitates protein sorting in the secretory pathway via cholesterol's ability to modify the membrane thickness [67]. This proposal has recently been reinforced by the observation that transmembrane domains of membrane proteins are not generic in nature, but are characterized by organelle-specific length between the early and late parts of the secretory pathway [68]. Membrane softening caused by low levels of cholesterol (see above; [65,66]) may play a role in the vesicle budding process associated with membrane traffic in Golgi and ER. Newly synthesized cholesterol is transported from ER, its site of synthesis, to the plasma membrane, predominantly by non-vesicular transport that bypasses the Golgi apparatus [69,70]. However, vesicular transport from ER through the Golgi could bring some nascent cholesterol to the plasma membrane involving the vesicle budding process. Cholesterol is known to modulate lipid–protein interactions by increasing the thickness of the lipid bilayer [71]. For example, it has been shown that POPC vesicles have a bilayer hydrophobic thickness of ~26 Å which increases to 30 Å in presence of 30 mol% cholesterol [72]. In fact, there is evidence that a gradient of cholesterol exists in Golgi and the membrane coming out of Golgi apparatus is believed to be thicker and having more cholesterol than the membrane entering from the ER. Since our results show that cholesterol dimerization is controlled by membrane thickness, this could imply that there is a gradient of cholesterol dimers across the Golgi apparatus.

An intriguing aspect of the transbilayer dimers is their sensitivity to membrane curvature (Fig. 3b). Many important cellular trafficking events (such as vesiculation, tubulation, pinching off of endocytic vesicles, and fusion of exocytic vesicles) are accompanied by sharp changes in membrane curvature [73,74]. The overall process of membrane trafficking and turnover is influenced by membrane curvature, e.g., vesicles budding off from ER or Golgi may have more surface curvature than their parent membrane. Curvature-induced membrane domains have earlier been reported to be responsible for differential sorting and trafficking of lipid analogues in CHO cells [75]. Some of the vesicles involved in cellular traffic, e.g., coated vesicles used in exocytosis, are quite small and are highly curved [76]. It is known that certain regions of sorting endosomes experience high curvature stress [75]. Interestingly, it has recently been reported that pH has a strong effect on membrane curvature [77], and the endosomal vesicles experience low (acidic) pH [78]. The modulation of transbilayer cholesterol dimerization by these cellular factors represents an interesting possibility. Interestingly, the detection of transbilayer cholesterol dimers has given rise to the intriguing possibility, that this could be a mechanism by which cholesterol-rich domains could be nucleated in the relatively cholesterol-poor Golgi membranes [79]. An interesting possibility is whether transbilayer cholesterol dimers could play an important role in coupling the two leaflets in the 'lipid raft' model.

The transbilayer tail-to-tail cholesterol organization could have a role in diseased states. Lipid peroxidation, a degenerative process correlated with aging and oxidative stress, has been reported to induce transbilayer domains of cholesterol at low concentrations of cholesterol, i.e., low cholesterol/phospholipid ratio [80]. The importance of cholesterol transbilayer domains in atherogenesis was previously demonstrated by Tulenko et al. [81]. In this study, small angle x-ray diffraction was used to examine arterial smooth muscle cell plasma membranes isolated from control and cholesterol-fed atherosclerotic rabbits. Beyond 9 weeks of cholesterol feeding, x-ray diffraction patterns demonstrated a lateral immiscible cholesterol domain with a bilayer thickness of 34 Å coexisting with the liquid crystalline (fluid) lipid bilayer (see Fig. 4a). The membrane thickness

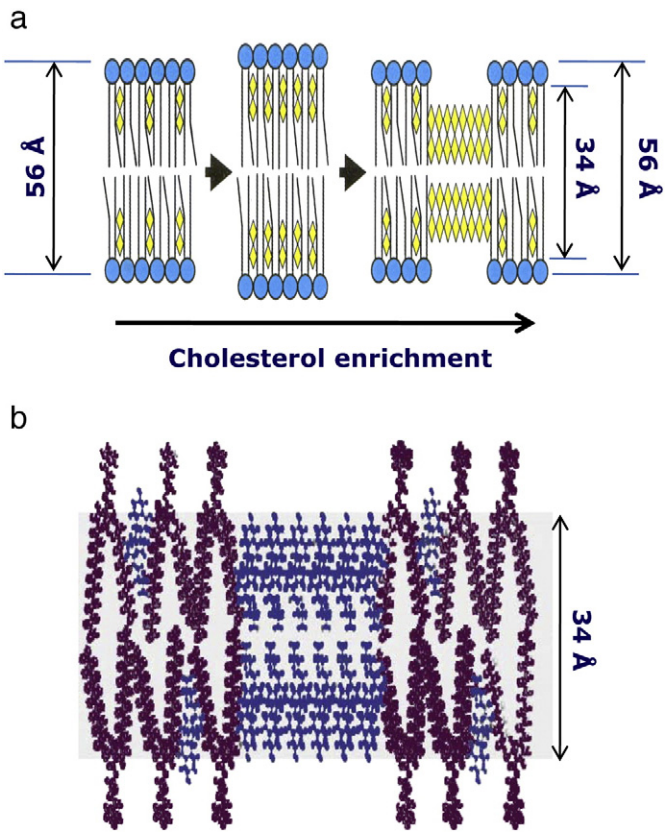

Fig. 4. Transbilayer cholesterol dimers in pathological conditions. (a) A schematic model showing the presence of transbilayer cholesterol dimers in arterial smooth muscle cell membranes in atherosclerotic rabbits. (b) Proposed model of transbilayer cholesterol dimers in the human ocular lens fiber cell plasma membranes. This type of cholesterol organization is more predominant in cataractous human lenses. See text for details. Adapted and modified from [81] and [84] with permission from American Society of Biochemistry and Molecular Biology.

of 34 Å corresponds to a tail-to-tail arrangement of cholesterol dimers as the length of an individual cholesterol molecule is ~17 Å [82,83]. This points out the possible involvement of the transbilayer tail-to-tail organization of cholesterol in atherogenesis. In addition, transbilayer cholesterol domains have been implicated in human ocular lens fiber cell plasma membranes, especially in cataractous condition [84–86]; see Fig. 4b).

As mentioned above, literature on cholesterol domains is very rich in case of membranes containing higher amounts of cholesterol. In this overall context, information on cholesterol domains formed in conditions of low cholesterol content is somewhat limited. Nonetheless, the unique organization exhibited by cholesterol, when present in relatively low concentrations, offers the possibility of providing novel insight into cellular sorting and disease processes.

Acknowledgements

Work in A.C.'s laboratory was supported by the Council of Scientific and Industrial Research, Govt. of India. Ar.C. thanks the Council of Scientific and Industrial Research for the award of a Senior Research Fellowship. A.C. is an Adjunct Professor at the Special Centre for Molecular Medicine of Jawaharlal Nehru University (New Delhi, India) and Indian Institute of Science Education and Research (Mohali, India), and Honorary Professor of the Jawaharlal Nehru Centre for Advanced Scientific Research (Bangalore, India). A.C. gratefully acknowledges J.C. Bose Fellowship (Dept. Science and Technology, Govt. of India). Some of the work described in this article was carried out by former members of A.C.'s group whose contributions are gratefully acknowledged. We thank Yamuna Devi Paila for help with the figures, and members of our laboratory for critically reading the manuscript.

References

[1] L. Liscum, K.W. Underwood, Intracellular cholesterol transport and compartmentation, J. Biol. Chem. 270 (1995) 15443–15446.
[2] K. Simons, E. Ikonen, How cells handle cholesterol, Science 290 (2000) 1721–1725.
[3] O.G. Mouritsen, M.J. Zuckermann, What's so special about cholesterol? Lipids 39 (2004) 1101–1113.
[4] J. Villalaín, Location of cholesterol in model membranes by magic-angle-sample-spinning NMR, Eur. J. Biochem. 241 (1996) 586–593.
[5] Y. Lange, T.L. Steck, Cholesterol homeostasis and the escape tendency (activity) of plasma membrane cholesterol, Prog. Lipid Res. 47 (2008) 319–332.
[6] K.E. Bloch, Sterol structure and membrane function, CRC Crit. Rev. Biochem. 14 (1983) 47–92.
[7] T. Róg, M. Pasenkiewicz-Gierula, I. Vattulainen, M. Karttunen, What happens if cholesterol is made smoother: importance of methyl substituents in cholesterol ring structure on phosphatidylcholine–sterol interaction, Biophys. J. 92 (2007) 3346–3357.
[8] S. Pöyry, T. Róg, M. Karttunen, I. Vattulainen, Significance of cholesterol methyl groups, J. Phys. Chem. B 112 (2008) 2922–2929.
[9] T. Róg, M. Pasenkiewicz-Gierula, Cholesterol effects on the phosphatidylcholine bilayer nonpolar region: a molecular simulation study, Biophys. J. 81 (2001) 2190–2202.
[10] S.A. Pandit, E. Jakobsson, H.L. Scott, Simulation of the early stages of nano-domain formation in mixed bilayers of sphingomyelin, cholesterol, and dioleylphosphatidylcholine, Biophys. J. 87 (2004) 3312–3322.
[11] P.L. Yeagle, Cholesterol and the cell membrane, Biochim. Biophys. Acta 822 (1985) 267–287.
[12] R. Bittman, Has nature designed the cholesterol side chain for optimal interaction with phospholipids? Subcell. Biochem. 28 (1997) 145–171.
[13] F. Schroeder, J.K. Woodford, J. Kavecansky, W.G. Wood, C. Joiner, Cholesterol domains in biological membranes, Mol. Membr. Biol. 12 (1995) 113–119.
[14] K. Simons, E. Ikonen, Functional rafts in cell membranes, Nature 387 (1997) 569–572.
[15] X. Xu, E. London, The effect of sterol structure on membrane lipid domains reveals how cholesterol can induce lipid domain formation, Biochemistry 39 (2000) 843–849.
[16] S. Mukherjee, F.R. Maxfield, Membrane domains, Annu. Rev. Cell Dev. Biol. 20 (2004) 839–866.
[17] O.G. Mouritsen, The liquid-ordered state comes of age, Biochim. Biophys. Acta 1798 (2010) 1286–1288.
[18] K. Simons, G. van Meer, Lipid sorting in epithelial cells, Biochemistry 27 (1988) 6197–6202.
[19] K. Simons, D. Toomre, Lipid rafts and signal transduction, Nat. Rev. Mol. Cell Biol. 1 (2000) 31–39.
[20] K. Simons, R. Ehehalt, Cholesterol, lipid rafts, and disease, J. Clin. Invest. 110 (2002) 597–603.
[21] J. Riethmüller, A. Riehle, H. Grassmé, E. Gulbins, Membrane rafts in host–pathogen interactions, Biochim. Biophys. Acta 1758 (2006) 2139–2147.
[22] T.J. Pucadyil, A. Chattopadhyay, Cholesterol: a potential therapeutic target in Leishmania infection? Trends Parasitol. 23 (2007) 49–53.
[23] M.S. Bretscher, S. Munro, Cholesterol and the Golgi apparatus, Science 261 (1993) 1280–1281.
[24] L. Orci, R. Montesano, P. Meda, F. Malaisse-Lagae, D. Brown, A. Perrelet, P. Vassalli, Heterogeneous distribution of filipin-cholesterol complexes across the cisternae of the Golgi apparatus, Proc. Natl Acad. Sci. USA 78 (1981) 293–297.
[25] R.A. Coxey, P.G. Pentchev, G. Campbell, E.J. Blanchette-Mackie, Differential accumulation of cholesterol in Golgi compartments of normal and Niemann-Pick Type C fibroblasts incubated with LDL: a cytochemical freeze-fracture study, J. Lipid Res. 34 (1993) 1165–1176.
[26] Y. Lange, Disposition of intracellular cholesterol in human fibroblasts, J. Lipid Res. 32 (1991) 329–339.
[27] K. Burger, G. Gimpl, F. Fahrenholz, Regulation of receptor function by cholesterol, Cell. Mol. Life Sci. 57 (2000) 1577–1592.
[28] T.J. Pucadyil, A. Chattopadhyay, Role of cholesterol in the function and organization of G-protein coupled receptors, Prog. Lipid Res. 45 (2006) 295–333.
[29] Y.D. Paila, A. Chattopadhyay, Membrane cholesterol in the function and organization of G-protein coupled receptors, Subcell. Biochem. 51 (2010) 439–466.
[30] G. van Meer, Lipid traffic in animal cells, Annu. Rev. Cell Biol. 5 (1989) 247–275.
[31] T.N. Estep, D.B. Mountcastle, R.L. Biltonen, T.E. Thompson, Studies on the anomalous thermotropic behavior of aqueous dispersions of dipalmitoylphosphatidylcholine–cholesterol mixtures, Biochemistry 17 (1978) 1984–1989.
[32] M.R. Vist, J.H. Davis, Phase equilibria of cholesterol/dipalmitoylphosphatidylcholine mixtures: ^{2}H nuclear magnetic resonance and differential scanning calorimetry, Biochemistry 29 (1990) 451–464.
[33] T.P.W. McMullen, R.N.A.H. Lewis, R.N. McElhaney, Differential scanning calorimetric study of the effect of cholesterol on the thermotropic phase behavior of a homologous series of linear saturated phosphatidylcholines, Biochemistry 32 (1993) 516–522.
[34] J.S. Harris, D.E. Epps, S.R. Davio, F.J. Kezdy, Evidence for transbilayer, tail-to-tail cholesterol dimers in dipalmitoylglycerophosphocholine liposomes, Biochemistry 34 (1995) 3851–3857.
[35] S. Mukherjee, A. Chattopadhyay, Membrane organization at low cholesterol concentrations: a study using 7-nitrobenz-2-oxa-1, 3-diazol-4-yl-labeled cholesterol, Biochemistry 35 (1996) 1311–1322.
[36] L.M.S. Loura, M. Prieto, Dehydroergosterol structural organization in aqueous medium and in a model system of membranes, Biophys. J. 72 (1997) 2226–2236.
[37] R. Rukmini, S.S. Rawat, S.C. Biswas, A. Chattopadhyay, Cholesterol organization in membranes at low concentrations: effects of curvature stress and membrane thickness, Biophys. J. 81 (2001) 2122–2134.
[38] M.B. Sankaram, T.E. Thompson, Modulation of phospholipid acyl chain order by cholesterol. A solid-state ^{2}H nuclear magnetic resonance study, Biochemistry 29 (1990) 10676–10684.
[39] M.B. Sankaram, T.E. Thompson, Cholesterol-induced fluid-phase immiscibility in membranes, Proc. Natl Acad. Sci. USA 88 (1991) 8686–8690.
[40] O. Albrecht, H. Gruler, E. Sackmann, Pressure-composition phase diagrams of cholesterol/lecithin, cholesterol/phosphatidic acid, and lecithin/phosphatidic acid mixed monolayers: a Langmuir film balanced study, J. Colloid Interface Sci. 79 (1981) 319–338.
[41] G. Gimpl, K. Gehrig-Burger, Cholesterol reporter molecules, Biosci. Rep. 27 (2007) 335–358.
[42] D. Wüstner, Fluorescent sterols as tools in membrane biophysics and cell biology, Chem. Phys. Lipids 146 (2007) 1–25.
[43] A. Chattopadhyay, Chemistry and biology of N-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl)-labeled lipids: fluorescent probes of biological and model membranes, Chem. Phys. Lipids 53 (1990) 1–15.
[44] S.S. Rawat, S. Mukherjee, A. Chattopadhyay, Micellar organization and dynamics: a wavelength-selective fluorescence approach, J. Phys. Chem. B 101 (1997) 1922–1929.
[45] S.S. Rawat, A. Chattopadhyay, Structural transition in the micellar assembly: a fluorescence study, J. Fluoresc. 9 (1999) 233–244.
[46] A. Chattopadhyay, S. Mukherjee, H. Raghuraman, Reverse micellar organization and dynamics: a wavelength-selective fluorescence approach, J. Phys. Chem. B 106 (2002) 13002–13009.
[47] K. Ray, R. Badugu, J.R. Lakowicz, Langmuir–Blodgett monolayers of long-chain NBD derivatives on silver island films: well-organized probe layer for the metal-enhanced fluorescence studies, J. Phys. Chem. B 110 (2006) 13499–13507.
[48] T.J. Pucadyil, S. Mukherjee, A. Chattopadhyay, Organization and dynamics of NBD-labeled lipids in membranes analyzed by fluorescence recovery after photobleaching, J. Phys. Chem. B 111 (2007) 1975–1983.
[49] M. Takahashi, M. Murate, M. Fukuda, S.B. Sato, A. Ohta, T. Kobayashi, Cholesterol controls lipid endocytosis through Rab11, Mol. Biol. Cell 18 (2007) 2667–2677.
[50] A. Chattopadhyay, E. London, Spectroscopic and ionization properties of N-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl)-labeled lipids in model membranes, Biochim. Biophys. Acta 938 (1988) 24–34.
[51] S. Lin, W.S. Struve, Time-resolved fluorescence of nitrobenzoxadiazole-aminohexanoic acid: effect of intermolecular hydrogen-bonding on non-radiative decay, Photochem. Photobiol. 54 (1991) 361–365.

[52] S. Fery-Forgues, J.-P. Fayet, A. Lopez, Drastic changes in the fluorescence properties of NBD probes with the polarity of the medium: involvement of a TICT state? J. Photochem. Photobiol. A 70 (1993) 229–243.
[53] S. Mukherjee, A. Chattopadhyay, A. Samanta, T. Soujanya, Dipole moment change of NBD group upon excitation studied using solvatochromic and quantum chemical approaches: implications in membrane research, J. Phys. Chem. 98 (1994) 2809–2812.
[54] A. Chattopadhyay, E. London, Parallax method for direct measurement of membrane penetration depth utilizing fluorescence quenching by spin-labeled phospholipids, Biochemistry 26 (1987) 39–45.
[55] B. Mitra, G.G. Hammes, Membrane-protein structural mapping of chloroplast coupling factor in asolectin vesicles, Biochemistry 29 (1990) 9879–9884.
[56] D.A. Kelkar, A. Chattopadhyay, Depth-dependent solvent relaxation in reverse micelles: a fluorescence approach, J. Phys. Chem. B 108 (2004) 12151–12158.
[57] S. Mukherjee, A. Chattopadhyay, Monitoring cholesterol organization in membranes at low concentrations utilizing the wavelength-selective fluorescence approach, Chem. Phys. Lipids 134 (2005) 79–84.
[58] A.L. McIntosh, B.P. Atshaves, H. Huang, A.M. Gallegos, A.B. Kier, F. Schroeder, Fluorescence techniques using dehydroergosterol to study cholesterol trafficking, Lipids 43 (2008) 1185–1208.
[59] J.J. Feher, L.D. Wright, D.B. McCormick, Studies of the self-association and solvent-association of cholesterol and other 3β-hydroxysteroids in nonpolar media, J. Phys. Chem. 78 (1974) 250–255.
[60] S. Mukherjee, A. Chattopadhyay, Wavelength-selective fluorescence as a novel tool to study organization and dynamics in complex biological systems, J. Fluoresc. 5 (1995) 237–246.
[61] A. Chattopadhyay, Exploring membrane organization and dynamics by the wavelength-selective fluorescence approach, Chem. Phys. Lipids 122 (2003) 3–17.
[62] A.P. Demchenko, Site-selective red-edge effects, Methods Enzymol. 450 (2008) 59–78.
[63] S. Haldar, A. Chattopadhyay, Dipolar relaxation within the protein matrix of the green fluorescent protein: a red edge excitation shift study, J. Phys. Chem. B 111 (2007) 14436–14439.
[64] A. Chattopadhyay, S. Mukherjee, Fluorophore environments in membrane-bound probes: a red edge excitation shift study, Biochemistry 32 (1993) 3804–3811.
[65] J. Lemmich, T. Hønger, K. Mortensen, J.H. Ipsen, R. Bauer, O.G. Mouritsen, Solutes in small amounts provide for lipid-bilayer softness: cholesterol, short-chain lipids, and bola lipids, Eur. Biophys. J. 25 (1996) 61–65.
[66] J. Lemmich, K. Mortensen, J.H. Ipsen, T. Hfnger, R. Bauer, O.G. Mouritsen, The effect of cholesterol in small amounts on lipid-bilayer softness in the region of the main phase transition, Eur. Biophys. J. 25 (1997) 293–304.
[67] H.R.B. Pelham, S. Munro, Sorting of membrane proteins in the secretory pathway, Cell 75 (1993) 603–605.
[68] H.J. Sharpe, T.J. Stevens, S. Munro, A comprehensive comparison of transmembrane domains reveals organelle-specific properties, Cell 142 (2010) 158–161.
[69] R.E. Soccio, J.L. Breslow, Intracellular cholesterol transport, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 1150–1160.
[70] E. Ikonen, Cellular cholesterol trafficking and compartmentalization, Nat. Rev. Mol. Cell Biol. 9 (2008) 125–138.
[71] V. Anbazhagan, D. Schneider, The membrane environment modulates self-association of the human GpA TM domain—implications for membrane protein folding and transmembrane signaling, Biochim. Biophys. Acta 1798 (2010) 1899–1907.
[72] F.A. Nezil, M. Bloom, Combined influence of cholesterol and synthetic amphiphilic peptides upon bilayer thickness in model membranes, Biophys. J. 61 (1992) 1176–1183.
[73] J. Zimmerberg, Are the curves in all the right places? Traffic 1 (2000) 366–368.
[74] M.M. Kozlov, Joint efforts bends membrane, Nature 463 (2010) 439–440.
[75] S. Mukherjee, T.T. Soe, F.R. Maxfield, Endocytic sorting of lipid analogues differing solely in the chemistry of their hydrophobic tails, J. Cell Biol. 144 (1999) 1271–1284.
[76] M. Aridor, W.E. Balch, Principles of selective transport: coat complexes hold the key, Trends Cell Biol. 6 (1996) 315–320.
[77] J.B. Lee, P.G. Petrov, H.-G. Döbereiner, Curvature of zwitterionic membranes in transverse pH gradients, Langmuir 15 (1999) 8543–8546.
[78] S. Mukherjee, R.N. Ghosh, F.R. Maxfield, Endocytosis, Physiol. Rev. 77 (1997) 759–803.
[79] M. Edidin, Lipid microdomains in cell surface membranes, Curr. Opin. Struct. Biol. 7 (1997) 528–532.
[80] R.F. Jacob, R.P. Mason, Lipid peroxidation induces cholesterol domain formation in model membranes, J. Biol. Chem. 280 (2005) 39380–39387.
[81] T.N. Tulenko, M. Chen, P.E. Mason, R.P. Mason, Physical effects of cholesterol on arterial smooth muscle membranes: evidence of immiscible cholesterol domains and alterations in bilayer width during atherogenesis, J. Lipid Res. 39 (1998) 947–956.
[82] B.M. Craven, Crystal structure of cholesterol monohydrate, Nature 260 (1976) 727–729.
[83] W.-G. Wu, L.-M. Chi, Conformational change of cholesterol side chain in lipid bilayers, J. Am. Chem. Soc. 113 (1991) 4683–4685.
[84] R.F. Jacob, R.J. Cenedella, R.P. Mason, Direct evidence for immiscible cholesterol domains in human ocular lens fiber cell plasma membranes, J. Biol. Chem. 274 (1999) 31613–31618.
[85] R.F. Jacob, R.J. Cenedella, R.P. Mason, Evidence for distinct cholesterol domains in fiber cell membranes from cataractous human lenses, J. Biol. Chem. 276 (2001) 13573–13578.
[86] M. Raguz, J. Widomska, J. Dillon, E.R. Gaillard, W.K. Subczynski, Characterization of lipid domains in reconstituted porcine lens membranes using EPR spin-labeling approaches, Biochim. Biophys. Acta 1778 (2008) 1079–1090.