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Alpha-Crystallin Association with the Model of Human and Animal Eye Lens-Lipid Membranes is Modulated by Surface Hydrophobicity of Membranes

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Abstract

Purpose: This research aims to probe the interaction of α-crystallin with a model of human, porcine, and mouse lens-lipid membranes.

Methods: Cholesterol/model of human lens-lipid (Chol/MHLL), cholesterol/model of porcine lens-lipid (Chol/MPLL), and cholesterol/model of mouse lens-lipid (Chol/MMLL) membranes with 0 to 60 mol% Chol were prepared using the rapid solvent exchange method and probe-tip sonication. The hydrophobicity near the surface of model lens-lipid membranes and α-crystallin association with these membranes were investigated using the electron paramagnetic resonance spin-labeling approach.

Results: With increased Chol content, the hydrophobicity near the surface of Chol/MHLL, Chol/MPLL, and Chol/MMLL membranes, the maximum percentage of membrane surface occupied (MMSO) by α-crystallin, and the association constant (K_a) decreased, showing that surface hydrophobicity of model lens-lipid membranes modulated the α-crystallin association with these membranes. The different MMSO and K_a for different model lens-lipid membranes with different rates of decrease of MMSO and K_a with increased Chol content and decreased hydrophobicity near the surface of these membranes suggested that the lipid composition also modulates α-crystallin association with membranes. Despite different lipid compositions, complete inhibition of α-crystallin association with model lens-lipid membranes was observed at saturating Chol content forming cholesterol bilayer domains (CBDs) with the lowest hydrophobicity near the surface of these membranes. The decreased mobility parameter with increased α-crystallin concentration suggested that membranes near the surface became less mobile due to α-crystallin association.

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Declaration of interest

The authors report no conflicts of interest.

The decreased mobility parameter and increased maximum splitting with increased Chol content suggested that membranes became less mobile and more ordered near the surface with increased Chol content.

Conclusions: This study suggested that the interaction of α-crystallin with model lens-lipid membranes is hydrophobic. Furthermore, our data indicated that Chol and CBDs reduce α-crystallin association with lens membrane, likely increase α-crystallin concentration in lens cytoplasm, and possibly favor the chaperone-like activity of α-crystallin maintaining lens cytoplasm homeostasis.

Keywords

α-crystallin; model lens-lipid membrane; hydrophobic interaction; cholesterol; lens cytoplasm homeostasis

Introduction

Approximately 40% of the eye lens protein is α-crystallin,[1,2] which works as a molecular chaperone[3,4] by preventing precipitation of denatured proteins and increasing tolerance to stress.[5] There are hydrophobic regions on the surface of α-crystallin.[6,7] The surface hydrophobicity of α-crystallin is believed to be significant for chaperone-like activity[7,8] because these hydrophobic regions may associate with exposed hydrophobic sites of denatured proteins, preventing their aggregation. The hydrophobic sites of two subunits of α-crystallin, i.e., αA- and αB-crystallin,[9,10] have been reported to play a crucial role in the chaperone-like activity.[11–13] Plater et al. reported that N-terminal phenylalanine-rich regions in αB-crystallin, which are primarily hydrophobic, are necessary for the chaperone-like activity.[14] In addition, the temperature-dependent experiments[8,15–19] provided robust supporting evidence for the hydrophobicity of α-crystallin playing a significant role in the chaperone-like activity. The increased temperature increased the chaperone-like activity of α-crystallin, mainly because heating caused structural changes in α-crystallin following increased exposure of additional hydrophobic sites.[8,15–19] Therefore, hydrophobic regions of α-crystallin are possibly crucial for maintaining lens transparency by preventing the aggregation of denatured proteins and cataract formation and progression.[20]

The most general cause of cataract, which causes blurred vision, is aging, in which the association of α-crystallin with the eye lens membrane increases continuously.[21–28] Water-soluble α-crystallin gradually depletes, becoming insoluble aggregates with age and onset of cataract;[23,25,29] however, more studies are needed to explore the nature of such insoluble aggregates.[30–34] An important factor in cataract development is α-crystallin aggregation.[35,36] Most water-insoluble crystallins, even in transparent lenses, are self-associated and remain associated with other crystallins within the core of the cytoplasm, whereas some are membrane-associated.[23] α-Crystallin associates with other lens proteins forming higher molecular weight complexes (HMWC) with aging.[37–39] Some HMWC may form in the cytoplasm away from the membranes, and others may associate with membranes,[29] perhaps as anchoring points. How these HMWC form, accompanied by light scattering and cataract formation[21,22,40] is still not well understood. A clinical study[24] showed a higher level of membrane-associated α-crystallin, accompanied by a

corresponding decline of soluble α-crystallin in lens cytoplasm, caused nuclear cataract formation and progression. There is a hypothesis that membrane-associated α-crystallin obstructs the membrane pores, forms a diffusion barrier, and contributes to nuclear cataracts formation.[25,26,41]

The studies on the association of α-crystallin with lens membranes[6,42–47] and lipid vesicles[42,48–53] have gained substantial attention. However, the exact nature of the association of α-crystallin with lens membranes and lipid vesicles remains unclear. A few earlier studies reported that the association of α-crystallin with the lens plasma membrane is affected by the ionic interaction between α-crystallin and membrane lipids.[6,54] The studies on the association of α-crystallin with bovine lens-lipid membranes[40,42,55] and synthetic lipid membranes[29,46,51,56,57] suggested a noncovalent association of α-crystallin to these lipid membranes. An earlier infrared spectroscopy study[58] and our recent electron paramagnetic resonance (EPR) studies[50,52,53] reported that polar headgroup regions of the lipids strongly influence the interaction of α-crystallin with membranes. A few studies reported that the surface hydrophobicity of α-crystallin affects the association of α-crystallin with membranes.[43,50–53,59] A study performed using resonance energy transfer reported a deep association of α-crystallin into the lens lipid vesicles, and the association increased with the increased preincubation temperature.[40] This is likely due to increased exposure of hydrophobic regions when α-crystallin was preincubated at a higher temperature,[40] indicating hydrophobic interaction of α-crystallin with lipid vesicles. A fluorescence study[60] showed that α-crystallin association with lens plasma membranes increased in acidic pH and with removed intrinsic membrane proteins; however, it did not depend on the ionic strength, implying the association of α-crystallin with membranes is through hydrophobic interaction. Our EPR spin-labeling studies also suggested that α-crystallin associates with lipid membranes,[50,52,53] and cholesterol-containing lipid membranes[28,53] through hydrophobic interactions. Likely due to the denaturation of α-crystallin in older lenses, the hydrophobic regions of α-crystallin become more exposed with increased association with lens lipids,[25] accompanied by light scattering and cataracts formation.

In this study, we varied Chol content from 0 to 60 mol% within cholesterol/model of human lens-lipid (Chol/MHLL), cholesterol/model of porcine lens-lipid (Chol/MPLL), and cholesterol/model of mouse lens-lipid (Chol/MMLL) membranes and monitored the hydrophobicity near the surface of these membranes and the influence of change in hydrophobicity to the percentage of membrane surface occupied (MSO) by α-crystallin and association constant (K_a) of α-crystallin association with these membranes using the EPR spin-labeling method. Chol content modulates the hydrophobicity of membranes.[61–64] In the eye lens membranes, dramatic changes in the Chol content occur with age and cataracts[65,66] and among species.[67,68] Moreover, dramatic changes in lipid composition in the eye lens membranes occur with age and cataracts[67,69–75] and among species.[67,69,76,77] The study reported in this paper examines the association of α-crystallin with the model lens-lipid membranes in a controlled Chol content and lipid composition and shows that such association is modulated by the surface hydrophobicity of membranes, suggesting hydrophobic interaction of α-crystallin with membranes.

Materials and methods

Materials

Cholesterol (Chol), egg sphingomyelin (SM), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylserine (POPS), and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). The cholesterol analog cholestane spin-label (CSL), bovine eye lens α-crystallin (C4163), HEPES, and sodium chloride (NaCl) were obtained from Sigma Aldrich (St. Louis, MO, USA). Bovine α-crystallin purchased from Sigma Aldrich was used without further purification. The average molecular weight of the α-crystallin subunit was determined to be 20.35 kDa based on the information αA = 19.8 kDa, αB = 22 kDa, and αA:αB = 3:1 from Sigma Aldrich.

Preparation of samples

The unique sphingolipids and phospholipids (PLs) compositions to prepare MHLL, MPLL, and MMLL membranes were taken from the previously reported study by Deeley et al.[76] We used 66% SM, 11% POPC, 8% POPS, and 15% POPE to prepare MHLL membrane. Similarly, 29% SM, 35% POPC, 21% POPS, and 12% POPE were used to prepare MPLL membrane, and 15% SM, 46% POPC, 17% POPS, and 17% POPE were used to prepare MMLL membrane. The 1 mol% CSL spin-label was maintained in mixed Chloroform solutions of Chol and lipids (SM, POPC, POPC, and POPE). The mixing ratios of Chol/MHLL were maintained at 0, 0.5, 1.0, and 1.5 and Chol/MPLL and Chol/MMLL were maintained at 0, 0.5, and 1.0. The detailed method to prepare small unilamellar vesicles (SUVs) using the rapid solvent exchange method[78–80] followed by probe tip-sonication was described in our previous studies.[50,52,53] α-Crystallin, model lens-lipid membranes, and mixtures of α-crystallin and model lens-lipid membrane samples were prepared in HEPES buffer containing 10 mM HEPES, 100 mM NaCl, pH 7.4. Chol plus lipids (SM and three PLs) concentration in membrane samples was maintained at 40 mM. The three PLs include POPC, POPS, and POPE.

α-Crystallin at varying concentrations from 0 to 52.6 μM was mixed with each of Chol/MHLL, Chol/MPLL, and Chol/MMLL membrane at 11.4 mM concentration of Chol plus lipids (SM and three PLs). The mixed samples in a total of 70 μL were incubated for 16 h at 37 °C with gentle shaking in an incubator (Corning, NY, USA) to allow saturable association of α-crystallin with membranes, as explained in our previous studies.[50,52,53]

EPR measurements

The incubated model lens-lipid membranes with varied Chol content and in the absence of α-crystallin were loaded into a 0.8 mm i.d. gas permeable methylpentene polymer (TPX) capillary[81] for EPR measurements at about −165 °C using X-band Bruker ELEXSYS 500 spectrometer. The z-component of the hyperfine interaction tensor (A_z) for CSL in model lens-lipid membranes at different Chol concentrations was measured from EPR spectra recorded with an incident microwave power of 2.0 mW and modulation amplitude of 2.0 G for samples frozen at about −165 °C.[62,63,82–84] The $2A_z$ is the measure of hydrophobicity.[62,63,82–84] A controlled flow of liquid nitrogen was used to maintain the

temperature at about −165 °C. The $2A_z$ value increases with the decrease in hydrophobicity around the nitroxide moiety of CSL.[62,63,84] As shown in Figure 1, the horizontal distance ($2A_z$) between the low and high field lines in EPR spectra of frozen samples at about −165 °C gives hydrophobicity.[62,82–84] The $2A_z$ value can be measured with a precision of ±0.25 G.

The incubated mixed samples (membranes and α-crystallin) were loaded into a 0.8 mm i.d. gas-permeable methylpentene polymer (TPX) capillary[81] for EPR measurements at 37 °C using an X-band Bruker ELEXSYS 500 spectrometer connected with the temperature-control accessories. EPR measurements were taken after thorough deoxygenation of samples by nitrogen gas. The same nitrogen gas controls the temperature. EPR spectra were recorded with an incident microwave power of 8.0 mW and modulation amplitude of 1.0 G. The detailed method to calculate the percentage of membrane surface occupied (MSO) by α-crystallin and the association constant (K_a) was described in our previous studies.[50,52,53]

Physical properties of model lens-lipid membranes after α-crystallin association

The physical properties, i.e., mobility parameter and maximum splitting, of model lens-lipid membranes after the association of α-crystallin were measured. The mobility parameter provides information about the mobility (dynamics) of CSL spin-label in membranes.[85] The maximum splitting provides information about the order of CLS spin-label in membranes.[62,83,86] Since the nitroxide moieties of CSL spin-label remain close to the surface of membranes, the mobility parameter and maximum splitting provide information about mobility and order, respectively, near the surface of membranes. The mobility parameter and maximum splitting were measured as explained in earlier studies.[50,52,53,62,83]

Statistics

All the data are expressed as mean ± standard deviation (SD) from three independent experiments. For the same model lens-lipid membrane with different Chol content, statistically significant differences between hydrophobicity values, between MMSO values, and between K_a values were determined using the Student's t-test with $p \leq 0.05$ as the significance criterion.

Results and discussion

Hydrophobicity near the surface of model lens-lipid membranes

Figure 1 displays representative EPR spectra of CSL in frozen solutions of Chol/MHLL membrane at Chol/MHLL mixing ratios 0 and 1.5 and shows the method of measuring $2A_z$ values. The higher $2A_z$ value represents the lower hydrophobicity near the surface of the membrane. Thus, an increase in $2A_z$ in spectra of CSL in Chol/MHLL membrane at Chol/MHLL mixing ratio of 1.5 compared to Chol/MHLL mixing ratio of 0 indicates that the membrane near the surface becomes less hydrophobic in the presence of Chol.

Figures 2A, B, and C display hydrophobicity near the surface of Chol/MHLL, Chol/MPLL, and Chol/MMLL membranes, respectively. These membrane samples were the same samples in the absence of α-crystallin that were used as a control in α-crystallin-membrane

association studies (see sections below). The almost equal $2A_z$ values for the MHLL, MPLL, and MMLL membranes in the absence of Chol suggested almost equal hydrophobicity near the surface of these model lens-lipid membranes. This result suggested that even with different lipid compositions in MHLL, MPLL, and MMLL membranes, the hydrophobicity near the surface of these membranes was approximately the same. The increased $2A_z$ with increased Chol/MHLL, Chol/MPLL, and Chol/MMLL mixing ratios suggested decreased hydrophobicity near the surface of these model lens-lipid membranes. This is because Chol moves the polar headgroups apart, increasing water penetration near the surface of these membranes.[84] Hydrophobicity decreased in a similar trend near the surface of Chol/MHLL, Chol/MPLL, and Chol/MMLL membranes with increased Chol content, suggesting that Chol content is a major factor modulating the hydrophobicity of membranes near the surface. The differences between the hydrophobicity values for each model lens-lipid membrane at different Chol content were statistically significant with $p \leq 0.05$. Previously, the EPR spin-labeling studies showed decreased hydrophobicity near the surface of Chol/POPC[62], Chol/SM[63], and Chol/POPS[64] multilamellar vesicles with increased Chol content.

Percentage of membrane surface occupied (MSO) by α-crystallin on model lens-lipid membranes

Figures 3A, B, and C display MSO as functions of α-crystallin concentration for Chol/MHLL, Chol/MPLL, and Chol/MMLL membranes, respectively. The MSO for these model lens-lipid membranes increased initially with increased α-crystallin concentration, suggesting the increased association of α-crystallin with these membranes. The MSO became constant above certain α-crystallin concentrations, suggesting that the association of α-crystallin with these membranes was saturable. Approximately 11%, 8.7%, and 7.8% MMSO for the MHLL, MPLL, and MMLL membranes in the absence of Chol represented that the amount of α-crystallin associated with these membranes followed the trends: MHLL > MPLL > MMLL. The MMSO values obtained for MHLL, MPLL, and MMLL membranes were comparable to the MMSO values obtained for individual and two-component lipid membranes.[50,52,53] Our previous EPR studies[50,52,53] and a fluorescence spectroscopy study[51] showed that the amount of α-crystallin associated with the SM membrane was higher than the PC membrane. The SM content used in the MHLL, MPLL, and MMLL membranes was 66%, 29%, and 15%, respectively, and the POPC content used in the MHLL, MPLL, and MMLL membranes was 11%, 35%, and 46%, respectively. Therefore, the highest amount of SM and the lowest amount of POPC in the MHLL membrane was likely why the amount of α-crystallin associated with the MHLL membrane was the largest. Similarly, the lowest amount of SM and the highest amount of POPC in the MMLL membrane was likely why the amount of α-crystallin associated with the MMLL membrane was the smallest. The MMSO for model lens-lipid membranes reported in this paper agree with the result that approximately 10% of α-crystallin associated with the PC vesicles reported by Mulders et al.[6]

The decreased MMSO for model lens-lipid membranes with increased Chol content and decreased hydrophobicity near the surface of these membranes suggested that the amount of α-crystallin associated with these membranes decreased. The differences between the MMSO values (Figure 3) for each model lens-lipid membrane at different Chol content

and hydrophobicity were statistically significant with $p \leq 0.05$. The results of this study agree with our previous results that MMSO decreased with increased Chol content in the cholesterol-containing individual lipid membranes.[53] Based on our earlier data of decreased hydrophobicity near the surface of Chol/POPC,[62] Chol/SM,[63] and Chol/POPS[64] multilamellar vesicles with increased Chol content, previously we proposed that decreased MMSO for unilamellar cholesterol-containing individual lipid membranes with increased Chol content might be due to the decreased hydrophobicity near the surface of these membranes.[53] However, the study reported in this paper clearly showed that increased Chol content decreased hydrophobicity near the surface of model lens-lipid membranes, accompanied by the decreased MMSO (see Figures 2 and 3). Therefore, this study showed that α-crystallin association with model lens-lipid membranes was modulated by the surface hydrophobicity of these membranes, suggesting hydrophobic interaction of α-crystallin with membranes.

The rate of decrease of MMSO for different model lens-lipid membranes with increased Chol content and decreased hydrophobicity near the surface of these membranes was different (see Figure 3). The MMSO decreased rapidly for Chol/MMLL membrane and slowly for Chol/MHLL membrane. Even though Chol content modulates the hydrophobicity near the surface of model lens-lipid membranes in a similar trend, the MMSO was zero for Chol/MHLL membrane at a mixing ratio of 1.5 (60 mol% Chol) and for Chol/MPLL and Chol/MMLL membranes at mixing ratios of 1.0 (50 mol% Chol). These results signify the importance of different lipid compositions of these model lens-lipid membranes. Phospholipid cholesterol domain (PCD) forms when cholesterol (Chol) saturates the membrane.[53,72,87,88] With a further increase in Chol content, cholesterol bilayer domains (CBDs) form within the PCD.[72,82,87,88] CBDs start to form above ~46 mol% Chol within Chol/HMLL, Chol/MPLL, and Chol/MMLL membranes. As described by us earlier,[89] the assumption used to estimate the formation of CBDs within the model lens-lipid membrane is the weighted sum of the individual Chol content values for each lipid (SM, POPC, POPS, and POPE), with the weight equal to the mol% of each lipid in the membrane. At 48, 50, 46, and 33 mol% Chol within SM, POPC, POPS, and POPE membranes, respectively, CBDs start to form.[89] The study reported in this paper and previous studies[62–64] showed increased Chol content within the membranes decreased hydrophobicity on the membrane surface. Moreover, CBDs have significantly lower hydrophobicity on the membrane surface than the surrounding PCD.[62] This may be why, irrespective of lipid composition, high Chol content and CBDs significantly decreased surface hydrophobicity of membranes resulting in MMSO to be zero in all these model lens lipid membranes (see Figure 3). Therefore, this study suggests that Chol and CBDs in the lens membrane decrease hydrophobicity near the surface of the membrane, accompanied by the decreased association of α-crystallin with the membrane.

Association constant (K_a) of α-crystallin association with model lens-lipid membranes

Figures 4A, B, and C display the K_a as functions of α-crystallin concentration for Chol/MHLL, Chol/MPLL, and Chol/MMLL membranes, respectively. The decreased K_a with increased Chol content and decreased hydrophobicity near the surface of model lens-lipid membranes suggested that the strength of α-crystallin association with these membranes

decreased. This result further showed that α-crystallin association with model lens-lipid membranes was modulated by the surface hydrophobicity of these membranes, suggesting hydrophobic interaction of α-crystallin with membranes. Cobb and Petrash et al.[60] used fluorescent tag (Alexa350) in α-crystallin and suggested a hydrophobic interaction of α-crystallin with plasma membranes using fluorescence approach. Tang et al.[40] used tryptophan of α-crystallin as the energy donor and fluorescence probe dansyl DHPE incorporated in lens cortex lipid vesicles as an energy acceptor and suggested a hydrophobic interaction of α-crystallin with lipid vesicles using a resonance energy transfer method. Tjondro et al.[90] showed that heat treatment of αA-crystallin produced larger oligomers with increased association with lipid monolayer. Such increased association[90] was likely facilitated by the increased exposure of hydrophobic sites of αA-crystallin upon heat treatment, suggesting the hydrophobic interaction of αA-crystallin with lipid monolayer. In the study reported in this paper, we used the EPR spin-labeling methods and measured the decreased surface hydrophobicity of membranes with increased Chol content. Our results showed that the decreased surface hydrophobicity of membranes accompanied the decreased association of α-crystallin with membranes, suggesting the hydrophobic interaction of α-crystallin with membranes.

The differences between the K_a values (Figure 4) for each model lens-lipid membrane at different Chol content and hydrophobicity were statistically significant with $p \leq 0.05$. The K_a values reported in this study agree with the K_a values reported for individual and two-component lipid membranes[50,52] as well as for cholesterol-containing lipid membranes.[53] Mulders et al.[6] reported the K_a of 7.69 μM^{-1} for the association of α-crystallin with alkali-washed lens plasma membranes containing intrinsic lens membrane proteins. The K_a values reported in this study are slightly less than those reported by Mulders et al.[6], likely because our model lens-lipid membranes consist of Chol and lipids only without intrinsic lens membrane proteins.

The K_a for the association of α-crystallin with model lens-lipid membranes in the absence of Chol followed the trends: K_a (MPLL) > K_a (MHLL) > K_a (MMLL) (see Figure 4). The K_a for α-crystallin association with the MPLL membrane was approximately two times larger than the MHLL membrane. However, the K_a for α-crystallin association with the MPLL membrane was approximately 3.5 times larger than the MMLL membrane. Like MMSO (Figure 3), the K_a decreased rapidly for Chol/MMLL membrane and slowly for Chol/MHLL membrane (Figure 4) with increased Chol content and decreased hydrophobicity near the surface of these membranes. The K_a became zero when the mixing ratios reached 1.5 (60 mol% Chol) for Chol/MHLL membrane and 1 (50 mol% Chol) for Chol/MPLL and Chol/MMLL membranes. The zero K_a represents no association of α-crystallin with these model lens-lipid membranes at these saturating Chol content with CBDs within these membranes and the lowest hydrophobicity near the surface of these membranes. The difference between K_a values (see Figure 4) and MMSO values (see Figure 3) with different rates of decrease of K_a and MMSO with increased Chol content and decreased hydrophobicity near the surface of model lens-lipid membranes were likely due to different lipid compositions in these membranes. The difference in lipid compositions in different model lens-lipid membranes may cause a difference in the capacity of the mixture of lipids to modulate the likely hydrophobic interaction between α-crystallin and these membranes, with the synergic effect

of size and charge of headgroup of lipid, hydrogen bonding between headgroups, and lipid curvature playing a crucial role. However, irrespective of the lipid composition of the model lens-lipid membranes, the MMSO and K_a became zero at saturating Chol content forming CBDs within these membranes with the lowest hydrophobicity near the surface of these membranes (see Figures 3 and 4). Therefore, results reported in this paper suggested that Chol and CBDs in the lens membrane decrease hydrophobicity near the surface of the membrane, inhibit α-crystallin association with the membrane, and likely increase the concentration of water-soluble α-crystallin in the lens cytoplasm favoring its chaperone function and maintaining lens cytoplasm homeostasis.

Physical properties of model lens-lipid membranes

Figures 5A, B, and C display the mobility parameter profiles as functions of α-crystallin concentration for Chol/MHLL, Chol/MPLL, and Chol/MMLL membranes, respectively. The mobility parameter of model lens-lipid membranes in the absence of Chol and α-crystallin followed the trends: MMLL > MPLL > MHLL, suggesting that the MMLL membrane has the largest and the MHLL membrane has the smallest mobility near the surface. The decreased mobility parameter of MHLL, MPLL, and MMLL membranes with increased α-crystallin concentration suggested that these membranes became less mobile near the surface due to α-crystallin association. Two significant changes in the mobility parameter of Chol/MHLL, Chol/MPLL, and Chol/MMLL membranes have been observed with increased Chol content and decreased hydrophobicity near the surface of these membranes. First, the mobility parameter of model lens-lipid membranes decreased with increased Chol content and decreased hydrophobicity near the surface of these membranes. With an increased Chol/MHLL mixing ratio from 0 to 1.5 for Chol/MHLL membrane, accompanied by decreased hydrophobicity near the surface of this membrane, the mobility parameter decreased significantly (see Figure 5A). Similarly, with increased Chol/MPLL and Chol/MMLL mixing ratio from 0 to 1.0 for Chol/MPLL and Chol/MMLL membranes, accompanied by decreased hydrophobicity near the surface of these membranes, the mobility parameter decreased significantly (see Figure 5B and C). Second, with increased Chol content and decreased hydrophobicity near the surface of model lens-lipid membranes, the decreased mobility parameter with increased α-crystallin concentration was less noticeable. This was because the amount of α-crystallin associated with model lens-lipid membranes became less with increased Chol content and decreased hydrophobicity near the surface of these membranes, resulting in a reduced ability of α-crystallin to decrease the mobility of these membranes near the surface. At a mixing ratio of 1.5 for Chol/MHLL membrane and 1 for Chol/MPLL and Chol/MMLL membranes, and with the lowest hydrophobicity near the surface of these membranes, the mobility parameter of these membranes remained constant with increased α-crystallin concentration. This was because such high Chol content with the formation of CBDs within model lens-lipid membranes significantly decreased hydrophobicity near the surface of these membranes, accompanied by complete inhibition of α-crystallin association with these membranes. Using the EPR spin-labeling approach, we observed a similar decrease in the mobility parameter of the individual and two-component lipid membranes[50,52] as well as cholesterol-containing lipid membranes[53] after α-crystallin association. Using fluorophore NBD-PE, which partitions near the surface of the membrane,

Borchman and Tang et al.[42] found that the mobility of bovine lens-lipid vesicles near the surface decreased with the α-crystallin association.

Intuitively, the strength of α-crystallin association (K_a) and the maximum amount of α-crystallin association with the membrane (MMSO) determine how rapidly the mobility parameter decreases and the total decrease in the mobility parameter, respectively. The larger the K_a, the rapid the mobility parameter decrease, and vice-versa. The larger the MMSO, the higher the total decrease in mobility parameter, and vice-versa. Because the K_a for the association of α-crystallin with the MPLL membrane was the largest and MMLL membrane was the smallest, the decrease in the mobility parameter was rapid for the MPLL membrane and was slow for the MMLL membrane. Because the MMSO was the largest for the MHLL membrane and the smallest for the MMLL membrane, the total decrease in the mobility parameter was higher for the MHLL membrane and lower for the MMLL membrane.

Figures 6A, B, and C display the maximum splitting profiles as functions of α-crystallin concentration for Chol/MHLL, Chol/MPLL, and Chol/MMLL membranes, respectively. The maximum splitting of model membranes in the absence of Chol and α-crystallin followed the trends: MHLL > MPLL > MMLL, implying that the MHLL membrane has the highest and the MMLL membrane has the lowest order near the surface. SM content is high in the MHLL membrane, and POPC content is high in the MMLL membrane. Our results imply that the lipid composition strongly modulates the order near the surface of model lens-lipid membranes. Previously, we observed higher order near the surface of the SM membrane than that of other PL membranes.[50,52,53] Moreover, SM content increases, and PC content decrease in the eye lens membrane during aging.[75,91] Our observation that high order near the surface of the MHLL membrane and low order near the surface of MMLL membrane supports the increased order of lens membranes with aging.[92,93] The same trends of the maximum splitting and MMSO in the absence of Chol in model lens-lipid membranes suggested that highly ordered membranes near the surface can facilitate more association of α-crystallin. Most interestingly, the maximum splitting of model lens-lipid membranes increased with increased Chol content, suggesting that Chol and CBDs increased the order of these membranes near the surface. Previously, we observed a similar increase in the maximum splitting of cholesterol-containing individual lipid membranes with increased Chol content.[53] No significant change in the maximum splitting of model lens-lipid membranes with increased α-crystallin concentration suggested that the association of α-crystallin with these membranes did not significantly change the order near the surface. Previously, except for the SM and SM/POPE membranes,[52] we observed no significant change in the maximum splitting of the individual, two-component lipid membranes,[50,52] and cholesterol-containing lipid membranes[53] with increased α-crystallin concentration.

EPR spectra for all Chol concentrations in model lens-lipid membranes showed the typical lipid bilayer spectra (spectra not shown) with only Chol-dependent changes. The Chol-dependent changes in EPR spectra observed were decreased mobility parameter (see Figure 5) and increased maximum splitting (see Fig. 6) with increased Chol concentration. We did not observe any distortions in the EPR line shapes at high Chol content with CBDs within the model lens-lipid membranes. For example, Figure 2C in our previous study[53] showed typical bilayer spectra with no distortions in the EPR line shapes, where SUVs were

prepared at Chol/SM* mixing ratio of 1.5 (60 mol% Chol). Moreover, as in our previous studies,[50,52,53] no significant changes in EPR signals have been observed for membranes with and without Chol incubated for 0 h and 16 h at 37 °C, indicating the stability of SUVs. All these observations ensured that our SUVs remained intact during the experiment.

Conclusions

Most importantly, this study showed that the association of α-crystallin with model lens-lipid membranes was modulated by the surface hydrophobicity of these membranes, suggesting hydrophobic interaction of α-crystallin with membranes. In addition, our results showed that the lipid composition strongly modulated the interaction of α-crystallin with model lens-lipid membranes. However, irrespective of the lipid composition, the high Chol content forming CBDs decreased hydrophobicity near the surface of model lens-lipid membranes, leading to complete inhibition of α-crystallin association with these membranes. Moreover, our results showed that the increased association of α-crystallin with model-lens lipid membranes decreased mobility near the surface of these membranes, and increased Chol content increased order near the surface of these membranes. The results reported in this paper suggested that Chol and CBDs in the eye lens membrane decrease the hydrophobicity near the surface of the membrane, inhibit the association of α-crystallin with the lens membranes, and likely increase α-crystallin concentration in the lens cytoplasm favoring its chaperone activity and maintaining lens cytoplasm homeostasis.

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Data availability statement

The data that support the findings of this study are available from the corresponding author, LM, upon reasonable request.

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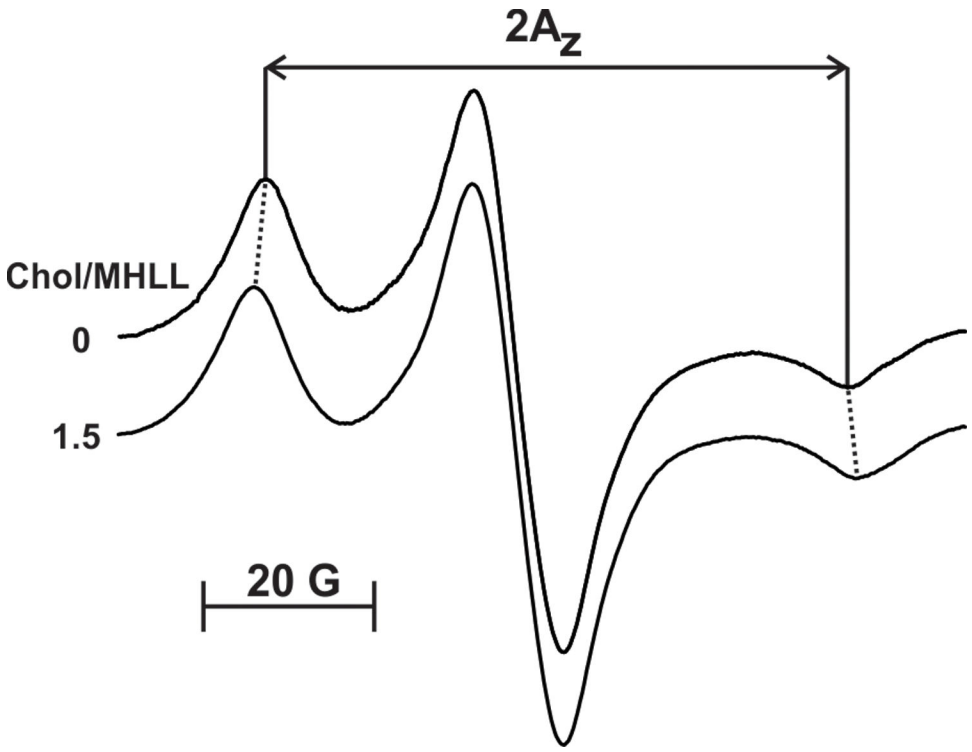

Figure 1.

EPR spectra of CSL in Chol/MHLL membrane at Chol/MHLL mixing ratios of 0 and 1.5 taken at about −165 °C canceling the effects due to motion. The dotted lines show that the $2A_z$ value increases with the increased Chol/MHLL mixing ratio from 0 to 1.5, representing decreased hydrophobicity near the surface of Chol/MHLL membrane with increased Chol content.

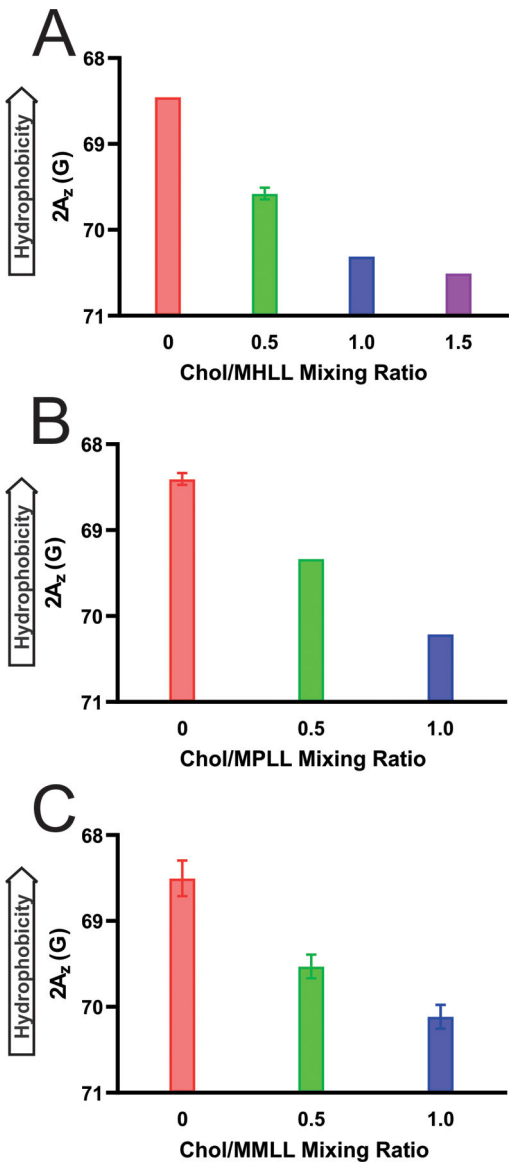

Figure 2.

(A), (B), and (C) The hydrophobicity ($2A_z$) near the membrane surface at different Chol content obtained using cholesterol analog spin-label (CSL) in Chol/MHLL, Chol/MPLL, and Chol/MMLL membranes, respectively. The decreased $2A_z$ represents increased hydrophobicity near the surface of membranes. The hydrophobicity near the surface of these model lens-lipid membranes decreased with increased Chol content.

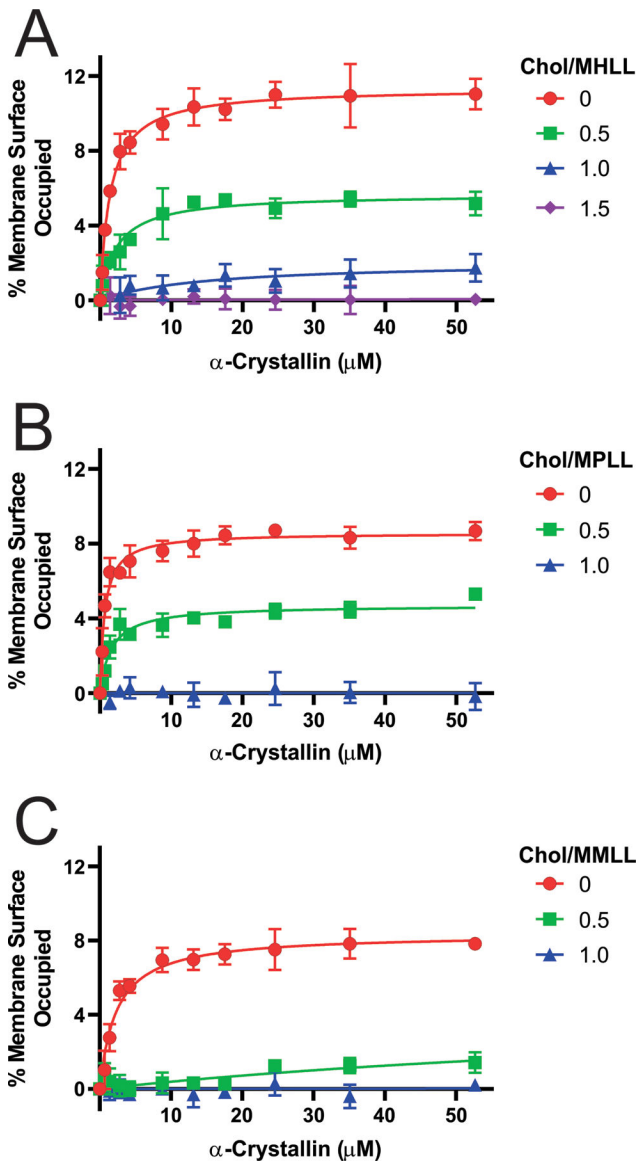

Figure 3.

(A), (B), and (C) The percentage of membrane surface occupied (MSO) as functions of α-crystallin concentration for Chol/MHLL, Chol/MPLL, and Chol/MMLL membranes, respectively. The MMSO decreased with increased Chol content and decreased hydrophobicity near the surface of these model lens-lipid membranes.

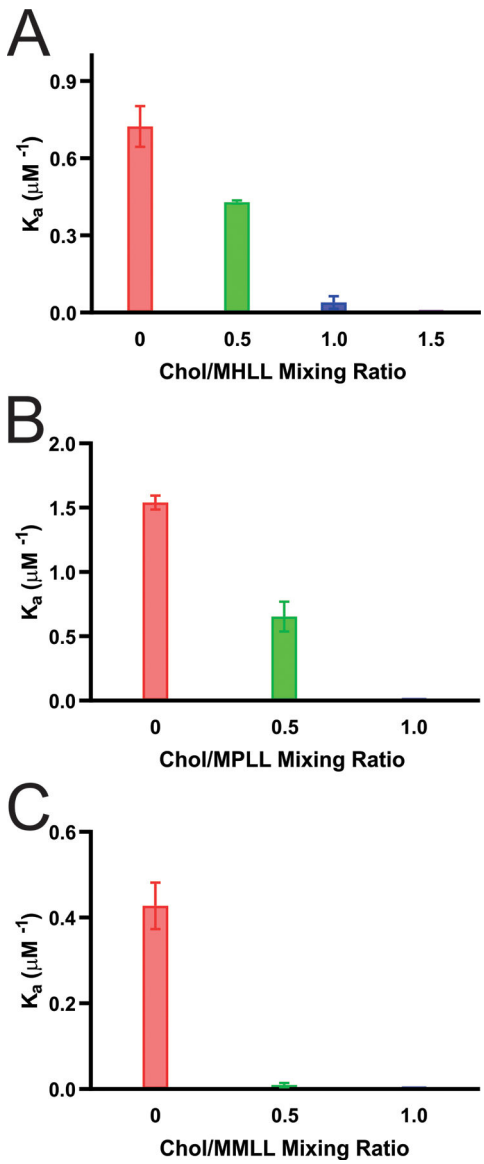

Figure 4.

(A), (B), and (C) The association constant (K_a) at different Chol content for Chol/MHLL, Chol/MPLL, and Chol/MMLL membranes, respectively. The K_a was calculated by fitting the MSO as functions of α-crystallin concentration data shown in Figure 3. The K_a decreased with increased Chol content and decreased hydrophobicity near the surface of these model lens lipid membranes.

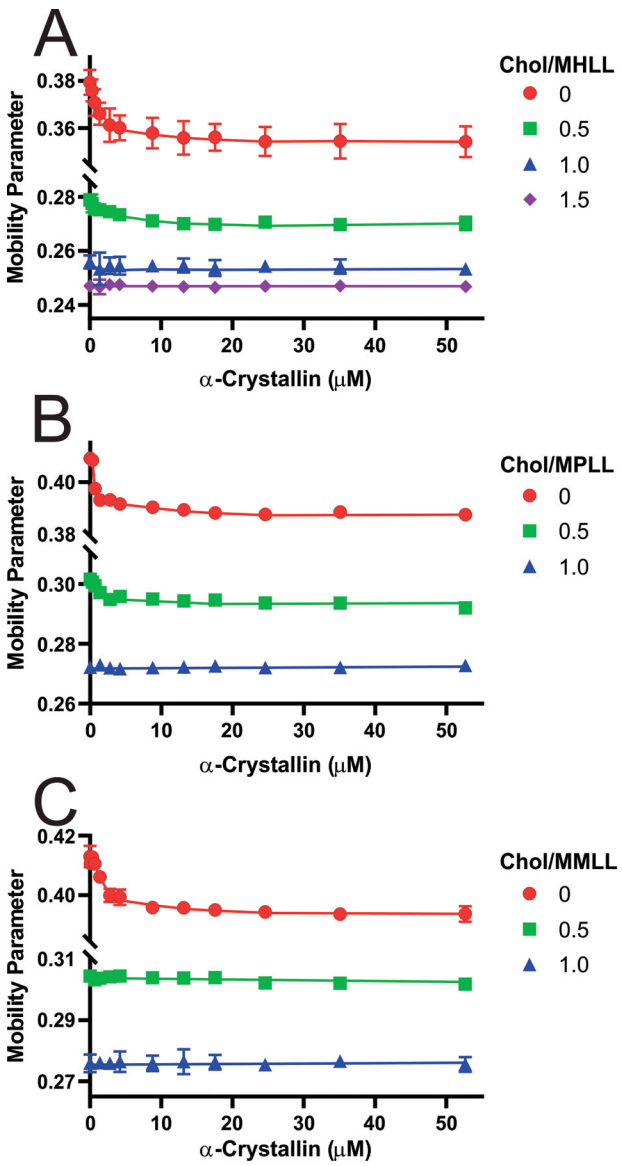

Figure 5.

(A), (B), and (C) Mobility parameter profiles as functions of α-crystallin concentration obtained at 37 °C using cholesterol analog spin-labels (CSL) in Chol/MHLL, Chol/MPLL, and Chol/MMLL membranes, respectively. The mobility parameter decreased with both increased α-crystallin association and Chol content.

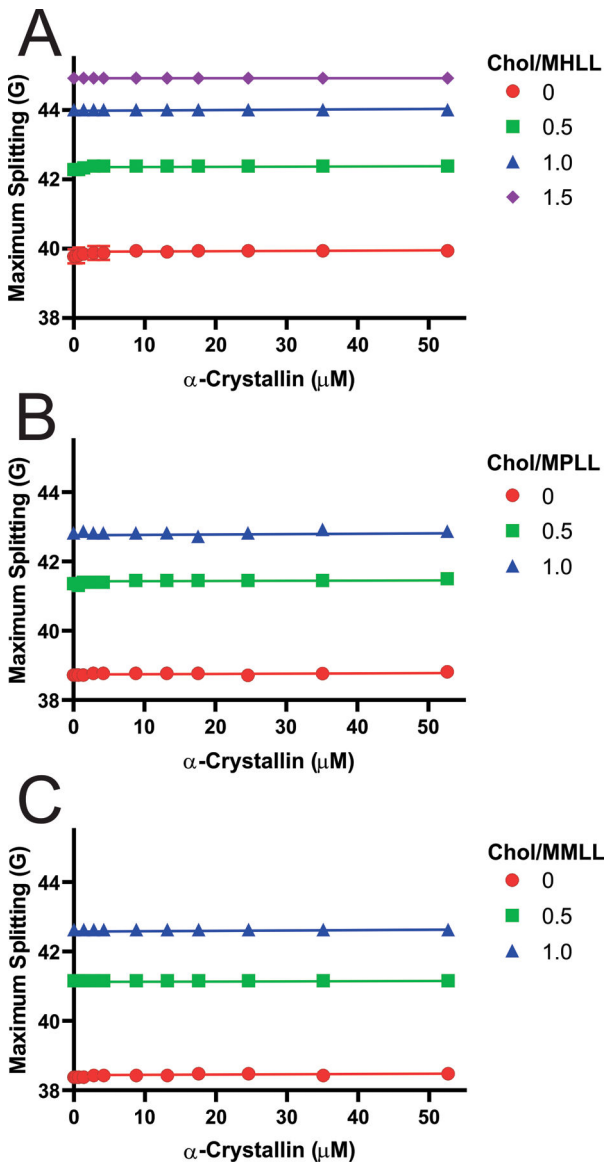

Figure 6.

(A), (B), and (C) Maximum splitting profiles as functions of α-crystallin concentration obtained at 37 °C using cholesterol analog spin-labels (CSL) in Chol/MHLL, Chol/MPLL, and Chol/MMLL membranes, respectively. The maximum splitting increased with increased Chol content.