

Oxysterol-Induced Rearrangement of the Liquid-Ordered Phase: A Possible Link to Alzheimer's Disease?

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SUPPORTING INFORMATION

Materials

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and cholesterol were obtained from Avanti Polar Lipids, Inc (Alabaster, AL). The oxysterols, 7 β -OH and 25-OH were obtained from Steraloids, Inc. (Newport, RI). Dihydrocholesterol was obtained from Alfa Aesar (Ward Hill, MA), and the fluorescent probe, Laurdan, was obtained from Ana Spec, Inc. (San Jose, CA). All chemicals were used as obtained.

Fluorescence experiments. Liposomes containing 20 or 40 mol% sterols (~200 nm diameter) and Laurdan (0.5 mol%) were prepared from thin lipid films using methods similar to those used in NMR experiments (see below). Liposomal dispersions were placed in sealed fluorescence cuvettes and the fluorescence of each sample then measured as a function of temperature using a Perkin Elmer LS50B Luminescence Spectrometer employing a temperature controlled cell holder. In all cases an excitation wavelength of 350 nm was used, along with a slit width of 2.5 nm. Fluorescence emissions were recorded from 350 to 600 nm. To correct for light scattering, the apparent fluorescence of similar dispersions that were devoid of Laurdan was subtracted from the apparent fluorescence of liposomes that did contain this fluorescent probe. Generalized Polarization (GP) values were calculated using the following equation: $GP = (I_{440} - I_{490}) / (I_{440} + I_{490})$, where I_{440} and I_{490} are fluorescence emission intensities at 440 and 490 nm respectively.

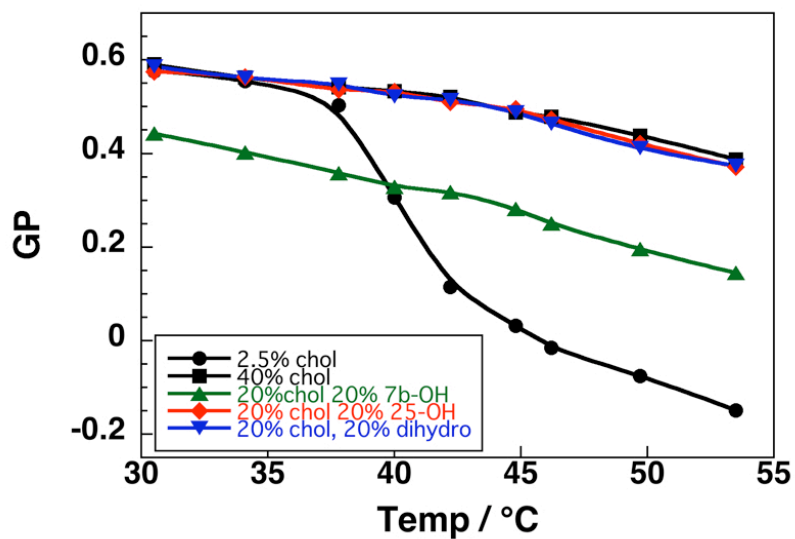


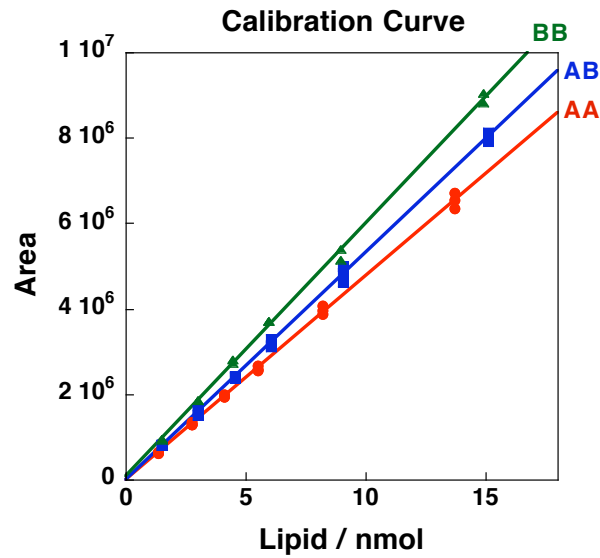
Figure S1. A comparison of GP values as a function of temperature for liposomes containing 20% cholesterol + 20% 7 β -OH (\blacktriangle), 20% cholesterol + 20% 25-OH (\blacklozenge), and 20% cholesterol + 20% dihydrocholesterol (\blacktriangledown) with ones containing 40 mol% cholesterol (\blacksquare) and 2.5% cholesterol (\bullet).

Nearest-Neighbor Recognition Analysis. In a typical liposome preparation, a thin film of lipid was prepared by evaporating a chloroform solution containing 0.30 μmol **AB** (or 0.15 μmol **AA** plus 0.15 μmol **BB**) and either 6.9 μmol of DPPC plus 4.5 μmol of sterols or 11.4 μmol of DPPC under a stream of argon. After drying the thin film overnight under reduced pressure (0.1 mm Hg), 2.0 mL of a 10 mM HEPES buffer (10 mM HEPES, 150 mM NaCl, 2 mM NaN_3 , 1 mM EDTA, pH = 7.4 at 45 °C) was added to the dried film. The mixture was vortex mixed for 30 s, incubated for 5 min at 60 °C, vortex mixed for an additional 30 s, and incubated for an additional 25 min at 60°C. The dispersion was then subjected to five freeze/thaw cycles (liquid nitrogen/60°C water bath), and extruded through a polycarbonate (Nuclepore) filter having pore diameters of 200 nm (11 times).

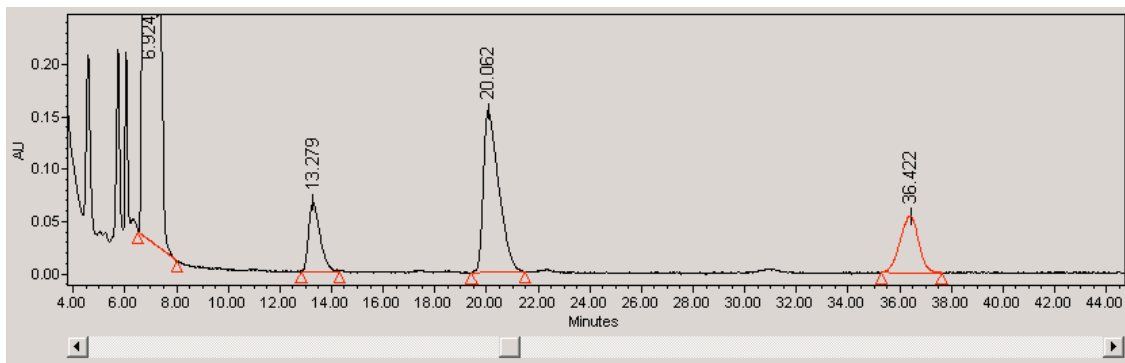
After the removal of oxygen from the system at 45 °C by purging with argon, thiolate-disulfide interchange reactions were then initiated by adding *threo*-dithiothreitol (typically 1.6 equiv) into 1650 μL of the dispersion. Aliquots (250 μL) were withdrawn as a function of time and the exchange reactions quenched by adding them to 7.3 μL of 10 M acetic acid at room temperature. The lipids were then quickly extracted into 1 mL of $\text{CHCl}_3/\text{MeOH}$ (2/1, v/v) and stored at -20°C. Just prior to analysis, a given sample was lyophilized and the lipids dissolved in 100 μL of solvent [i.e., HPLC mobile phase/ CHCl_3 (80/20, v/v)]. This solution was then immediately analyzed by HPLC using a C18 reverse phase column and a mobile phase that was composed of 10 mM tetrabutylammonium acetate in ethanol/water/hexane (76/13/10, v/v/v) using a flow rate of 0.9 mL/min. The column was maintained at 31°C and the components were monitored at 203 nm using a Waters 996-photodiode-array detector.

To confirm the equilibrium was reached, samples were withdrawn as a function of time or product mixtures that were derived from homodimers were compared with those derived from the corresponding heterodimer. It should be noted that NNR experiments carried out with liposomes prepared from homodimers tend to give K values that are slightly lower than for those made from the corresponding heterodimer. The reason for this small difference is a likely consequence of a distribution of the homodimers among the liposomes that is not perfectly random. Since NNR experiments using the heterodimer must contain an equimolar amount of **A** and **B** in every leaflet, the NNR data are more reliable. Thus, K values from the time course experiments using heterodimers are given in the text.

Calibration Curves. The exchangeable lipid dimers, **AA**, **AB**, and **BB**, were calibrated by HPLC. Varying amounts of exchangeable lipids were mixed with the HPLC mobile phase and injected to HPLC system. Peak areas were then plotted against the amount of lipid that was injected.



HPLC Chromatogram. Typical HPLC chromatogram is shown below. The retention times for dimers of **AA**, **AB** and **BB** were ca. 13 min, 20 min and 36 min, respectively.



lane	Retention Time (min)	Area ($\mu\text{V} \cdot \text{sec}$)	% Area	Height (μV)	Int Type	Amount	Units	Peak Type	Peak Codes
	6.924	23989456	68.27	806856	bb			Unknown	
	13.279	2027114	5.77	66410	bb			Unknown	
	20.062	6403163	18.22	154353	bb			Unknown	
	36.422	2721446	7.74	54426	bb			Unknown	

Values of K . Values of K were calculated from peak areas from the HPLC chromatograms using calibration curves; K values were calculated from the following equation: $K=[\mathbf{AB}]^2/([\mathbf{AA}]\times[\mathbf{BB}])$.

Raw data from NNR experiments

40% cholesterol (time course)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from hetero dimer	0	0.00	22.64	0.00	–
	20	4.92	14.85	4.98	9.00
	20	5.08	15.12	5.11	8.81
	24	5.15	15.53	5.26	8.90
	24	5.22	15.50	5.24	8.78
	28	4.98	15.12	4.99	9.21
	28	5.08	15.20	5.07	8.97
				average	8.95
			std	0.15	

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from hetero dimer	0	0.00	22.98	0.00	–
	21	5.25	15.61	5.22	8.88
	21	5.35	16.05	5.30	9.09
	24	5.29	15.88	5.35	8.91
	24	5.23	15.78	5.25	9.08
	28	5.01	14.90	5.07	8.74
	28	5.07	15.05	5.09	8.79
				average	8.91
			std	0.14	

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from homo dimer	0	12.33	0.00	12.73	–
	21	5.39	15.67	5.58	8.17
	21	5.48	15.64	5.57	8.01
	24	5.40	15.45	5.57	7.94
	24	5.43	15.54	5.61	7.92
	28	5.13	14.68	5.15	8.15
	28	4.93	14.09	5.05	7.98
				average	8.03
			std	0.11	

average (hetero & homo) 8.47
std (hetero & homo) 0.48

30% cholesterol, 10% 7b-OH (time course)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from hetero dimer	0	0.00	22.76	0.00	–
	20	4.77	15.21	4.90	9.90
	20	4.81	15.30	4.86	10.01
	24	4.94	15.30	4.83	9.81
	24	4.78	15.09	4.87	9.79
	28	4.54	14.47	4.61	10.02
	28	4.48	14.29	4.53	10.07
				average	9.93
				std	0.12

30% cholesterol, 10% 7b-OH

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from hetero dimer	0	0.00	16.62	0.00	–
	24	3.56	10.99	3.48	9.72
	24	3.70	11.76	3.78	9.89
	24	3.89	11.93	3.84	9.54
				average	9.72
				std	0.18

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from homo dimer	0	10.29	0.00	10.66	–
	24	4.41	13.36	4.85	8.36
	24	4.54	13.56	4.98	8.14
	24	4.62	13.74	5.04	8.11
				average	8.20
				std	0.14

average (hetero & homo) 8.96

std (hetero & homo) 0.84

20% cholesterol, 20% 7b-OH (time course)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from hetero dimer	0	0.00	23.33	0.00	–
	20	4.31	14.52	4.34	11.29
	20	4.43	14.37	4.51	10.36
	24	4.41	14.66	4.57	10.65
	24	4.39	14.75	4.55	10.89
	28	4.20	14.08	4.33	10.92
	28	4.23	14.11	4.41	10.69
				average	10.80
				std	0.32

20% cholesterol, 20% 7b-OH

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from hetero dimer	0	0.00	17.97	0.00	–
	24	3.65	12.02	3.89	10.17
	24	3.61	12.33	3.95	10.65
	24	3.75	12.52	4.05	10.32
				average	10.38
				std	0.25

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from homo dimer	0	9.09	0.00	9.67	–
	24	3.71	12.66	4.38	9.86
	24	3.80	12.91	4.50	9.72
	24	4.00	12.69	4.45	9.05
				average	9.54
				std	0.43

average (hetero & homo) 9.96

std (hetero & homo) 0.55

10% cholesterol, 30% 7b-OH (time course)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from hetero dimer	0	0.00	23.61	0.00	–
	20	4.54	15.67	4.62	11.69
	20	4.72	15.97	4.78	11.30
	24	4.70	15.95	4.71	11.51
	24	4.69	16.13	4.78	11.59
	28	4.25	14.83	4.36	11.86
	28	4.30	15.26	4.58	11.81
				average	11.63
				std	0.21

10% cholesterol, 30% 7b-OH

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from hetero dimer	0	0.00	17.70	0.00	–
	24	3.88	12.43	3.83	10.41
	24	3.86	13.13	3.94	11.34
	24	4.01	13.82	4.11	11.56
				average	11.11
				std	0.61

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from homo dimer	0	9.95	0.00	10.22	–
	24	4.33	13.75	4.51	9.67
	24	4.28	13.75	4.55	9.70
	24	4.47	14.48	4.64	10.10
				average	9.82
				std	0.24

average (hetero & homo) 10.46

std (hetero & homo) 0.82

20% cholesterol (time course)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from hetero dimer	0	0.00	25.38	0.00	–
	20	6.12	13.55	6.08	4.94
	20	6.12	13.84	6.18	5.07
	24	6.39	13.91	6.42	4.71
	24	6.43	14.17	6.39	4.89
	28	5.97	13.24	6.21	4.73
	28	6.13	13.31	6.04	4.79
			average	4.85	
			std	0.14	

20% cholesterol

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from homo dimer	0	10.75	0.00	11.11	–
	24	6.16	12.65	6.27	4.14
	24	6.05	12.74	6.36	4.22
	24	5.62	11.66	5.98	4.05
			average	4.13	
			std	0.09	
			average (hetero & homo)	4.45	
			std (hetero & homo)	0.36	

10% cholesterol, 10% 7b-OH (time course)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from hetero dimer	0	0.00	24.08	0.00	–
	20	5.36	13.47	5.48	6.18
	20	5.41	13.45	5.29	6.32
	24	5.60	13.82	5.67	6.01
	24	5.52	13.74	5.62	6.09
	28	5.67	14.15	5.70	6.19
	28	5.66	13.75	5.68	5.88
				average	6.11
				std	0.15

10% cholesterol, 10% 7b-OH

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from hetero dimer	0	0.00	20.44	0.00	–
	24	4.76	12.18	4.91	6.36
	24	4.77	11.72	4.69	6.14
	24	4.94	12.37	5.03	6.16
				average	6.22
				std	0.12

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from homo dimer	0	11.08	0.00	11.74	–
	24	5.46	12.27	6.16	4.48
	24	5.29	11.69	5.75	4.49
	24	5.49	12.16	6.03	4.46
				average	4.48
				std	0.01

average (hetero & homo) 5.35

std (hetero & homo) 0.96

20% cholesterol, 20% 25-OH (time course)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from hetero dimer	0	0.00	23.47	0.00	–
	20	5.11	14.60	5.22	7.98
	20	5.00	14.53	5.10	8.27
	24	5.17	14.81	5.13	8.27
	24	5.12	14.61	5.08	8.20
	28	5.28	15.06	5.33	8.05
	28	5.22	14.79	5.22	8.04
				average	8.14
				std	0.13

20% cholesterol, 20% 25-OH

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from hetero dimer	0	0.00	16.50	0.00	–
	24	3.53	10.13	3.67	7.91
	24	3.50	10.08	3.66	7.93
	24	3.88	11.23	4.13	7.86
				average	7.90
				std	0.04

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from homo dimer	0	9.24	0.00	9.88	–
	24	3.96	11.46	4.40	7.54
	24	4.05	11.74	4.60	7.40
	24	4.29	12.42	4.93	7.30
				average	7.41
				std	0.12

average (hetero & homo) 7.66

std (hetero & homo) 0.28

30% cholesterol, 10% 25-OH (time course)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from hetero dimer	0	0.00	22.83	0.00	–
	20	4.81	14.46	5.03	8.65
	20	4.77	14.26	4.94	8.64
	24	4.83	14.40	5.07	8.47
	24	4.74	14.34	5.00	8.68
	28	4.84	14.24	5.00	8.37
	28	4.88	14.30	5.02	8.34
				average	8.52
				std	0.15

10% cholesterol, 30% 25-OH (time course)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from hetero dimer	0	0.00	23.93	0.00	–
	20	5.17	14.47	5.29	7.67
	20	5.24	14.68	5.23	7.86
	24	5.24	14.41	5.19	7.64
	24	5.20	14.58	5.28	7.73
	28	5.07	14.22	5.06	7.90
	28	5.09	14.08	5.12	7.61
				average	7.74
				std	0.12

20% cholesterol, 20% dihydrocholesterol (time course)

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from hetero dimer	0	0.00	24.35	0.00	–
	20	5.02	14.84	4.92	8.92
	20	4.93	14.76	4.88	9.06
	24	5.11	15.58	5.16	9.21
	24	4.97	15.06	4.88	9.36
	28	4.97	15.12	5.01	9.19
	28	4.91	14.89	4.91	9.20
			average	9.16	
			std	0.15	

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from hetero dimer	0	0.00	24.58	0.00	–
	21	5.18	15.16	4.95	8.97
	21	5.13	15.27	5.14	8.84
	24	5.27	15.53	5.21	8.78
	24	5.36	15.98	5.42	8.80
	28	5.26	15.58	5.21	8.86
	28	5.27	15.65	5.19	8.96
			average	8.87	
			std	0.08	

	Time (h)	AA (nmol)	AB (nmol)	BB (nmol)	K
from homo dimer	0	12.10	0.00	12.45	–
	21	5.17	14.95	5.50	7.87
	21	5.07	15.25	5.38	8.52
	24	5.23	15.43	5.56	8.18
	24	5.21	15.44	5.43	8.43
	28	5.04	14.78	5.45	7.94
	28	5.11	14.91	5.38	8.09
			average	8.17	
			std	0.26	

average (hetero & homo)	8.52
std (hetero & homo)	0.41