tBid and cardiolipin

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Publication date:
2007

tBid and cardiolipin - trying an insight to the interplay of cell apoptosis key players in a simple model system

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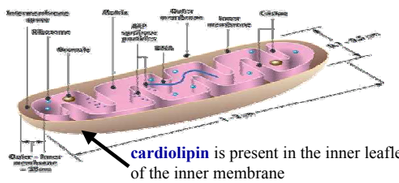
Introduction

The abundant presence of cardiolipin (CL) in the inner leaflet of inner mitochondrial membranes [1] has given rise to the suspicion that this lipid play be an essential role in triggering cell apoptosis, possibly by mechanically destabilizing the host membrane and thus enhancing the effect of the tBid apoptosis protein [2,3,4]. Therefore the mechanical effect of the presence of CL in model bilayer membranes was investigated by a combination of micromechanical deformation studies by vesicle aspiration (MA) and by differential calorimetry (DSC). At CL concentrations above 10% (mol/mol) vesicle formation was unsuccessful hinting towards a preference of non-lamellar phases. Yet the induction of hexagonal phases was not observed in the low contents regime (CL<5%). From the initial scans of the fresh samples, a phase diagram was constructed (CL <5%) that exhibits a continuous increase of melting temperatures with CL contents and that is accompanied by a loss in transition collaborativity. The calorimetry data are not easy to interpret due to the continuous chemical decay of the system upon hydrolysis.

Giant unilamellar vesicles as used for micromanipulation were stable for hours as judged from their shear appearance; light scattering on extruded vesicles revealed a conflicting result and showed a preference of stable radii of ~80-100nm independent on the initial preparation radius. The high shearing forces during extrusion may make use of a general instability induced by CL and kick the system into a favourite curvature conformation. The presence of CL seems to make membranes more expandable at essentially constant limiting tension. As the protein adsorbs to the interface, the expansion modulus is apparently increased by the presence of CL. We interpret this as a formation of patches of protein-lipid clusters that in effect reduce the amount of expandable fluid membrane area. The rupture tension falls significantly, both in the presence and absence of CL, as soon as tBid is present on the outer vesicle membrane.

Apoptosis model system chosen: protein and model membrane



cardiolipin is present in the inner leaflet of the inner membrane

protein: tBid

- 15kDa C-terminal fragment
- precursor: Bid
- tBid translocates to mitochondria
- major role in apoptosis
- suspicion: cardiolipin essential

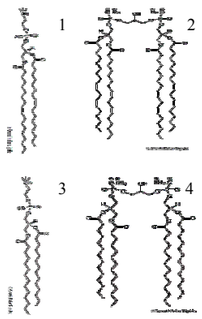
Lipid: mixture of phosphatidylcholine with cardiolipin

Micromanipulation studies:
major compound: DOPC (1)
minor compound: 6,9-tetra-linoleyl-cardiolipin (H-CL) (2)

Vesicle System:
electroswelling to GUVs

DSC studies
major compound: DMPC (3)
minor compound: 1,1',2,2'-tetramyristoyl cardiolipin (TM-CL) (4)

Vesicle System:
extrusion to SUVs (nominally ~200nm)



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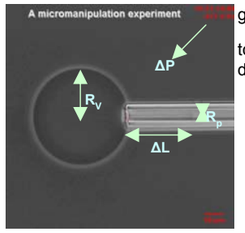
[5] http://www.avantilipids.com/TechnicalDetectionMethodsForThinLayerChromatography.htm

This work was supported in part by the Danish National Research Foundation via MEMPHYS - Center for Biomembrane Physics. B. K. is grateful to Serono Pharmaceutical Corp. for providing the protein.
Visit MEMPHYS - Center for Biomembrane Physics on the web at http://www.memphys.sdu.dk

Micromechanical Studies



goal: probe the mechanical effect of tBid inGUVs

total apparent fractional area change (α) due to applied excess tension ($\Delta\tau_I$)

$$\alpha = \left(\frac{\Delta A}{A_0}\right) = \frac{k_B T}{8\pi k_c} \ln\left(\frac{k_c \pi^2}{\Delta\tau_I a^2}\right) + \frac{\Delta\tau_I}{K_a}$$

isotropic excess tension

$$\Delta\tau_I = \frac{\Delta P R_P}{2 - 2R_P / R_0}$$

area increase

$$\Delta A = 2\pi R_p \Delta L \left(1 - \frac{R_p}{R_0}\right)$$

results:

1. area expansion modulus

system	number of vesicles n	buffer conditions	vesicle transfer status T/n-T	c_{tBid} [nM]	area expansion modulus K_a±SD [mN/m]	result after testing statistical relevance
pure DOPC	6	B_2	n-T	0	210±27	the presence of CL significantly reduces the area expansion modulus
DOPC:H-CL 95/5	36	B_2	n-T	0	170±40	
pure DOPC	9	B_1	T	0	205±56	administration of tBid has no significant effect on the areas expansion modulus in pure DOPC
pure DOPC	7	B_1	T	29	189±59	
DOPC:H-CL 95/5	10	B_2	T	0	153±27	the area expansion modulus is apparently increased on administration of tBid to vesicles of mixed DOPC/CL
DOPC:H-CL 95/5	10	B_2	T	29	221±24	

2. rupture tension

system	n	B.C	transfer status	c_{tBid} [nM]	σ_{rt} ±SD [mN/m]	Statistical testing
Pure DOPC	15	B_0	n-T	0	10,2±2,6	no difference
95/5	9	B_0	n-T	0	11,5±1,4	
Pure DOPC	9	B_1	T	0	7,2±2,4	rupture tension significantly decreased in the presence of tBid
Pure DOPC	10	B_1	T	29	5,0±0,9	
95/5	8	B_2	T	0	6,9±1,3	rupture tension significantly decreased in the presence of tBid
95/5	9	B_2	T	29	4,4±0,8	

buffer system	nomenclature
150mOSM Sucrose solution intra vesicular/ 150mOSM Glucose solution extra vesicular	B_0 conditions
150mOSM Sucrose solution intra vesicular/ 150mOSM Glucose-Hepes buffer extra vesicular	B_1 conditions
150mOSM Sucrose-Hepes buffer intra vesicular/ 150mOSM Glucose-Hepes buffer solution extra vesicular	B_2 conditions

Morphological Stability

results:

Sample	extrusion pore radius	hydrodynamic radius R_h [nm]	hydrodynamic radius R_h [nm]
		fresh	1 day old
Pure DMPC	200nm	83,4 (59,0-142,0)	76,5 (57,5-114,5)
	5000nm	387 (258-774)	359 (241-704)
DOPC:H-CL 98,7/1,3	200nm	71,6 (55,3-101,6)	71,2 (55,5-99,3)
	5000nm	106 (71,6-203,7)	104 (69,8-203,9)
DOPC:H-CL 95/5	200nm	74,5 (58,2-103,6)	74,1 (57,4-104,6)
	5000nm	81,7 (57,9-139,0)	80 (57,1-133,3)

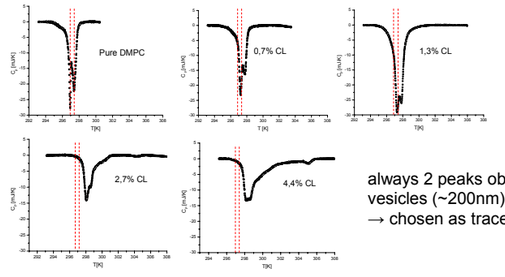
broad distributions obtained

the presence of cardiolipin causes a fast decrease of initially extruded radius towards an increased curvature

sizes are stable in time

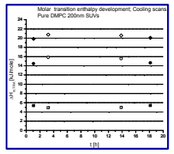
Differential Scanning Calorimetry

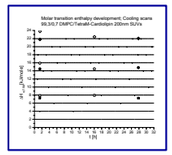
results:



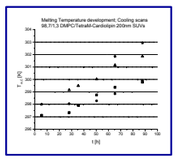
always 2 peaks observed for small vesicles (~200nm) of DMPC
→ chosen as tracer of CL-effects

Pure DMPC, 200nm SUVs	n	Peak 1	Peak 2	
$T_{m,C}$ ± SD [K]	4	296,8 ±0,10	297,0 ±0,09	
$T_{m,H}$ ± SD [K]	4	297,0 ±0,11	297,2 ±0,10	
$\Delta H_{mTRA,C}$ ± SD [kJ/mole]	4	5,2 ±0,26	15,1 ±0,67	
$\Delta H_{mTRA,H}$ ± SD [kJ/mole]	4	5,6 ±0,27	14,6 ±0,59	Total
$\Delta H_{mTRA,T,C}$ ± SD [kJ/mole]	4			20,3 ±0,55
$\Delta H_{mTRA,T,H}$ ± SD [kJ/mole]	4			20,2 ±0,46



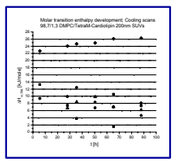


increase of molar enthalpy for the low enthalpy peak / low temperature peak headgroup melting observed separately? **CL enhancing headgroup interaction**?



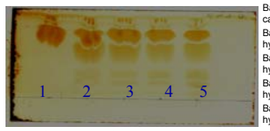
in the course of time development of more phases, and resulting modification of enthalpies measured

→ does CL induce instabilities?
size instabilities?
chemical instabilities?



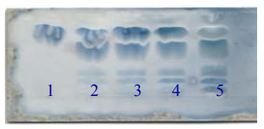
Chemical Stability

results:



iodine staining: Iodine stains all organic material

Band 1: fresh 1,1',2,2'-tetramyristoyl cardiolipin (TM-CL)
Band 2: 1 day old TM-CL, aged hydrated
Band 3: 2 day old TM-CL, aged hydrated
Band 4: 5 day old TM-CL, aged hydrated
Band 5: 10 day TM-CL, aged hydrated



phosphate staining: stains all compounds blue, that contain phosphate [5]

→ cardiolipin chemically decays in the course of time, most probably due to hydrolysis there is most probably a **mixture of CL originating components** in life systems that contain CL

Conclusion

The chosen model system exhibits inherent instabilities

- cardiolipin is chemically instable: aged samples must therefore be excluded from the investigations
- membranes containing cardiolipin are more easily expanded
- the rupture tension of a membrane is not modified by the presence of low amounts of cardiolipin
- **tBid** does not measurably modify the expansion modulus of pure DOPC membranes but does apparently **increase their resistance to expansion when cardiolipin is present**
- **tBid manifests itself in membranes, both with and without cardiolipin by a decrease in the rupture tension**
- proposed model: **chain mechanism** - **tBid seems to provide rupture sites / makes membranes less sustainable of stress**
  – cardiolipin makes membranes more susceptable to tBid / enhances the the number of rupture sites