EFFECT OF THE OXIDIZED PHOSPHOLIPID PALMITOYL-OXOVALEROYL PHOSPHATIDYLCHOLINE (POVPC) ON THE RAFT-LIKE DOMAIN FORMATION IN GIANT UNILAMELLAR VESICLES

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ABSTRACT

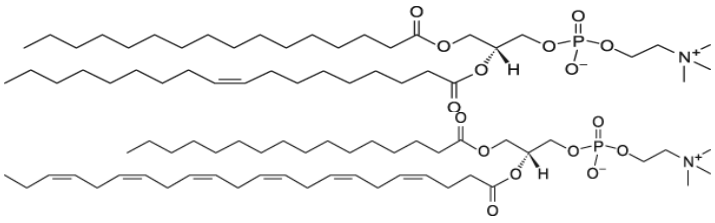
Fluorescence microscopy experiments were carried out to study the alterations in lateral membrane organization induced by the oxidized phospholipid palmitoyl-oxo-valeroyl-phosphocholine (POVPC) in giant unilamellar vesicles (GUVs). The vesicles were composed of ternary lipid mixtures of phosphatidylcholines (PC)/sphingomyelin (SM)/cholesterol (CHOL) (33/33/34 mol/mol/mol) mimicking the formation of raft-like domains within the membrane. Two hetero-acid glycerophosphocholines, palmitoyl-oleoyl phosphatidylcholine (POPC) and palmitoyl-docosahexaenoyl phosphatidylcholines (PDPC), were used to examine the effect of POVPC on the raft-like domain formation as function of the degree of fatty acid unsaturation.

THE AIM OF THIS STUDY:

In this work we have investigated the role of POVPC molecules in the lateral organization of plasma membrane lipids and formation of raft-like microdomains which serve as signal platforms within the cell membrane.

MATERIALS AND METHODS:

GUVs were prepared by electroformation method. Briefly, the vesicles, composed of PCs/SM/CHOL with and without POVPC, were formed at 45°C in 1mM Hepes buffer (pH 7.4). To visualize the lipid phase separation, the fluorescent marker Rhodamine PE at 1mol % was added to each lipid mixture. GUVs were observed by fluorescence microscopy using Zeiss Axiovert 135 microscope equipment.

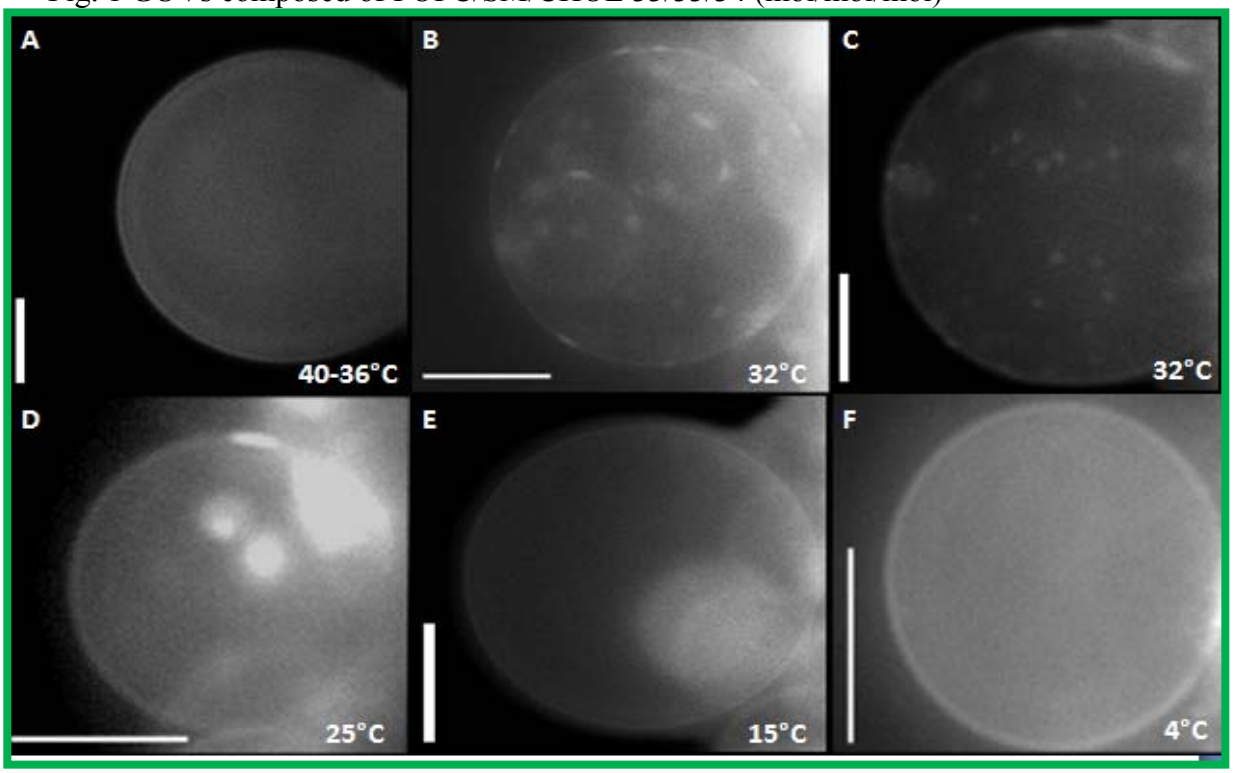


Palmitoyl-oleoyl phosphatidylcholine (16:0-18:1 PC)
POPC contains one double bond in oleoyl acid

Palmitoyl-docosahexaenoyl phosphatidylcholine (16:0-22:6 PC)
PDPC contains six double bonds in DHA

RESULTS:

Fig. 1 GUVs composed of POPC/SM/CHOL 33/33/34 (mol/mol/mol)



- Homogeneous appearance in 40-36°C temperature interval
- Below 32°C vesicles exhibited Ld (liquid-disordered)/ Lo (liquid-ordered) phase coexistence
- Further temperature decrease (25°C) caused enlargement of the bright domains as their number was reduced
- At temperatures below 15°C vesicles became homogenous ones

Fig. 2 GUVs composed of 33/33/34 POPC/SM/CHOL + POVPC in different mol %

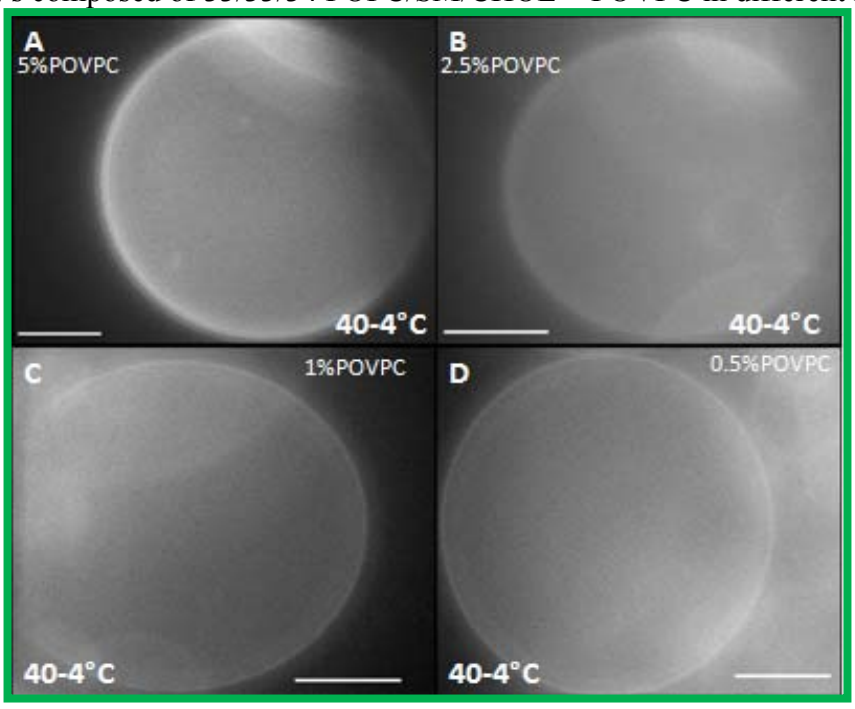
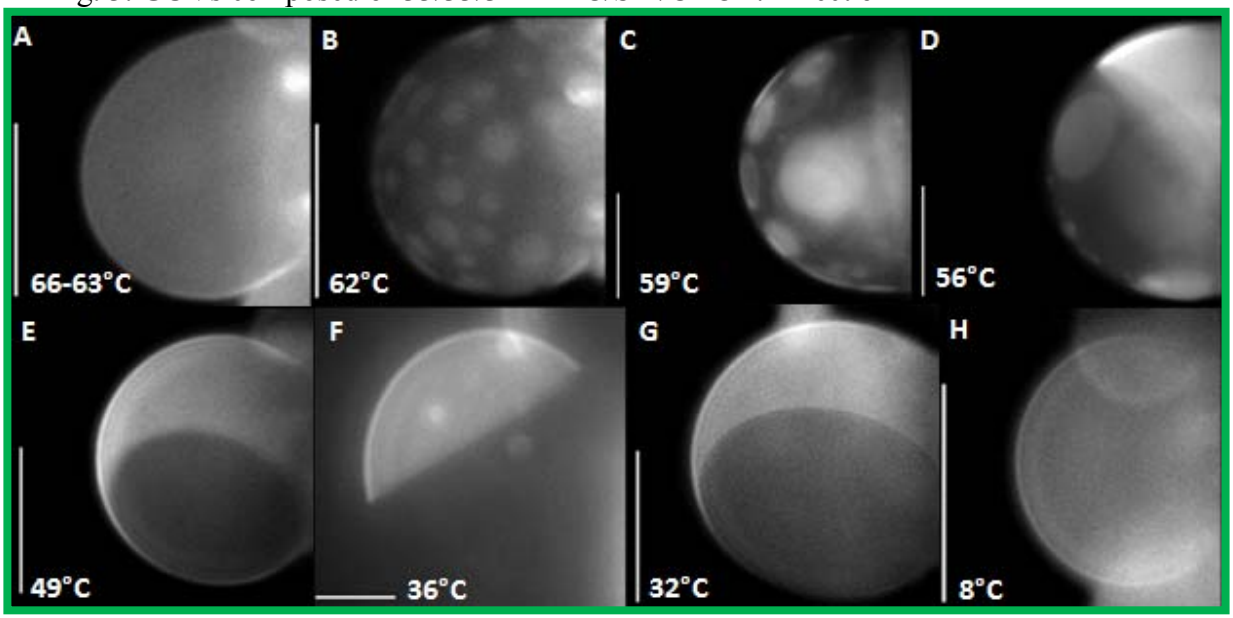
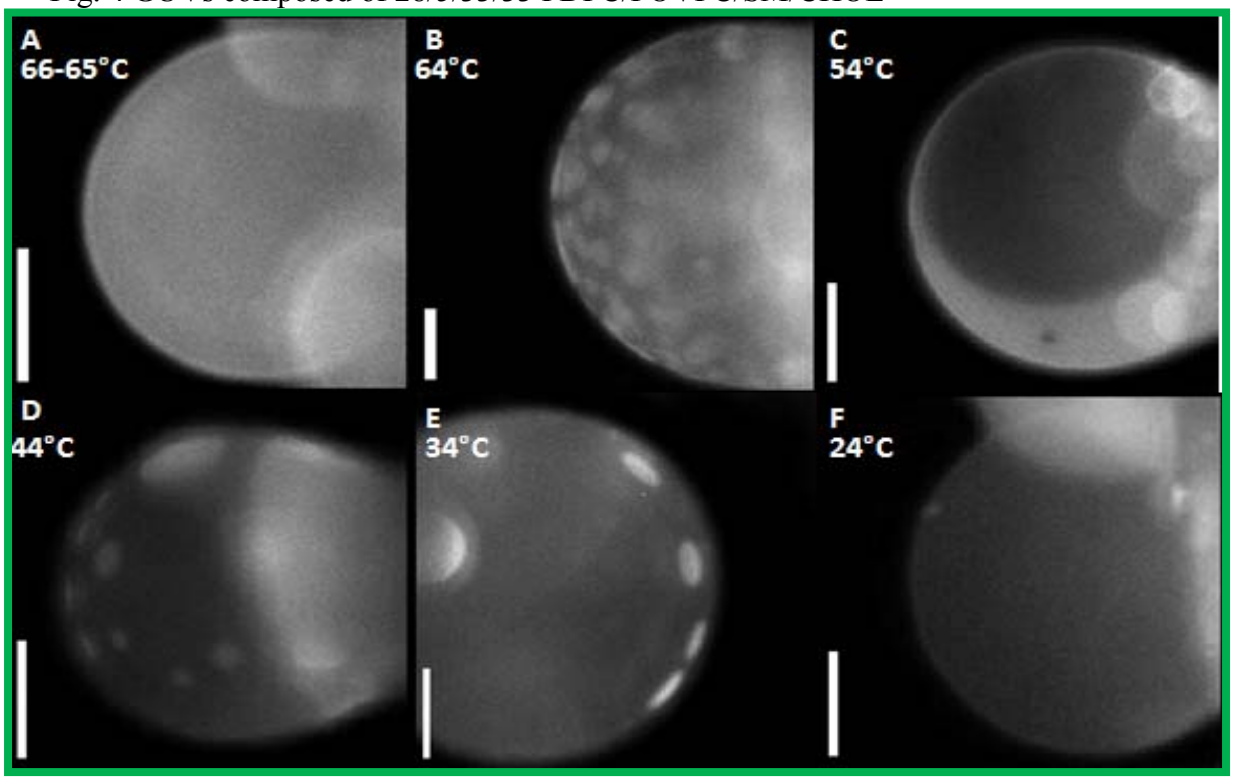


Fig. 3. GUVs composed of 33/33/34 PDPC/SM/CHOL: Effect of DHA



- Ld-homogeneous phase within the 60-63°C temperature interval
- Ld/Lo phase separation and percolation at 62°C
- Temperature decrease caused fusion and enlargement of the bright Ld domains (fig. 3C, D)
- At temperatures below 50°C, the fraction of Ld phase progressively increased
- At lower temperatures of 8°C, phase separation was not observed

Fig. 4 GUVs composed of 28/5/33/33 PDPC/POVPC/SM/CHOL

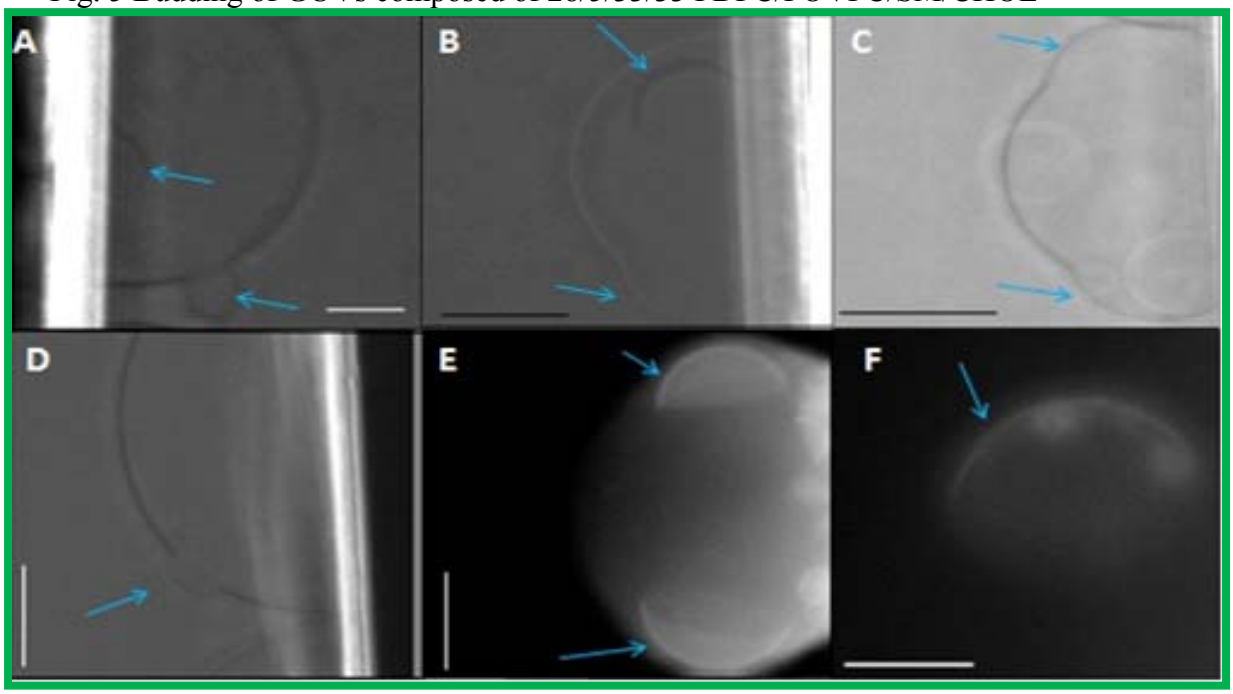


Phase separation of PDPC/SM/CHOL + 5mol % POVPC

- Homogeneous GUVs within the temperature range of from 66 to 65°C
- At 64°C phase percolation was observed (Ld domains on continuous Lo phase)

- Phase behavior changed at 54°C: the ratio in fractions of two coexisting phases was about 1/1
- Further temperature decrease led to gradual increase of the Lo domains fraction
- Below 24°C vesicles showed clear Lo phase

Fig. 5 Budding of GUVs composed of 28/5/33/33 PDPC/POVPC/SM/CHOL



The presence of 5 mol% POVPC induced budding and fission of the Ld domains at 36°C as it is shown here above.

Such phenomenon was not observed at lower concentrations of POVPC.

CONCLUSIONS:

- The addition of only 0.5 mol % of POVPC to POPC/POVPC/SM/CHOL mixture was enough to convert the heterogeneous membrane into a homogeneous one.
- The effect of POVPC in the presence of docosahexaenoic acid (DHA), containing 6 double bonds, in PDPC/POVPC/SM/CHOL mixture is completely different leading to higher temperature of raft-like domain formation.
- POVPC (5 mol %) induced Ld domains budding and fission from vesicles in continuous Lo phase at physiological temperature. It implies that oxidized POVPC molecules within DHA containing environment promote the processes of vesiculation which are very important for the physiological functions of the plasma membrane (protein sorting and transcytosis).

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