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ENZYMATIC DIGESTION OF HETEROGENEOUS PLANT GALACTOLIPID ASSEMBLIES BY PANCREATIC LIPASE RELATED-PROTEIN 2 (PLRP2)

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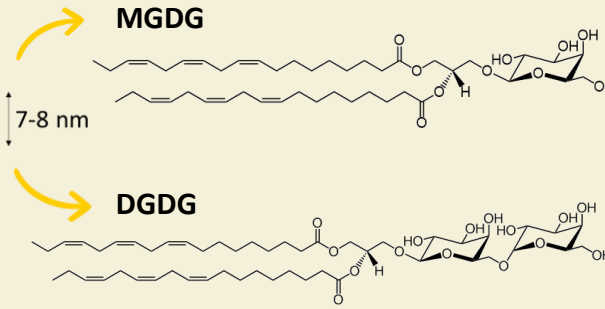
Kergomard et al., Biochimie 2022 - under review

Context

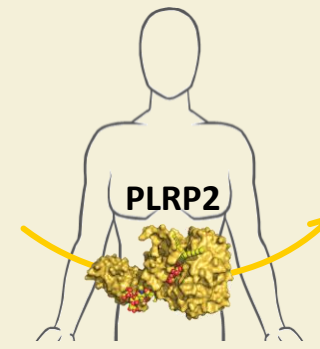


PLRP2 is responsible for the **effective hydrolysis of galactolipids** in human digestive system, leading to the production of **essential ω 3 fatty acids**. Besides chemical conditions, the PLRP2 adsorption has also been shown to be **modulated by the physical environment** of the substrate. Yet, PLRP2 adsorption and enzymatic activity has not been deeply investigated on biomimetic heterogeneous vegetal membranes containing **galactolipids**.

Photosynthetic plant membranes



Degradation and adsorption in the human digestive system



Free fatty acids
(ALA, EPA, DHA)
(C18:3, C20:5, C22:6)

Essential ω 3
(2.2 g/day, ANSES)

Sahaka et al, 2020

Objectives: to understand the **impact of the physical environment** on the adsorption and lipolytic activity of PLRP2 onto **heterogeneous biomimetic vegetal membrane** of increasing complexity using complementary biophysical tools (tensiometry, ellipsometry, atomic force microscopy) and *in vitro* static digestion.

Material and methods

*brassicasterol₁₀/campesterol₄₀/ β -sitosterol₄₀

Model systems

- 1 GL**
MGDG/DGDG
60:40 mol%
Homogeneous
- 2 GL/DPPC/pS**
MGDG/DGDG/DPPC/pS*
27:18:45:10 mol%
Heterogeneous
- 3 MGDG/DGDG/SQDG/PG**
MGDG/DGDG/SQDG/PG
56:24:10:10 mol%
Biomimetic

Molecular scale (nm)

Interfacial characterization
in Langmuir trough



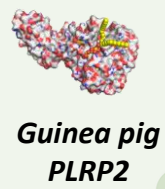
Lipid monolayers

Assembly scale (μ m)

Static *in vitro* model
(mL) - Bulk

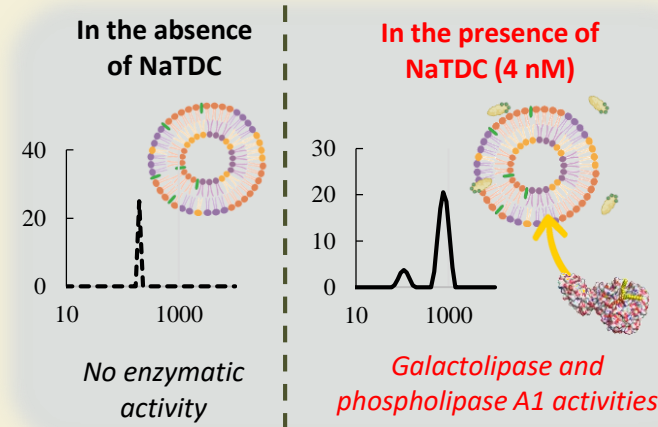


Giant liposomes

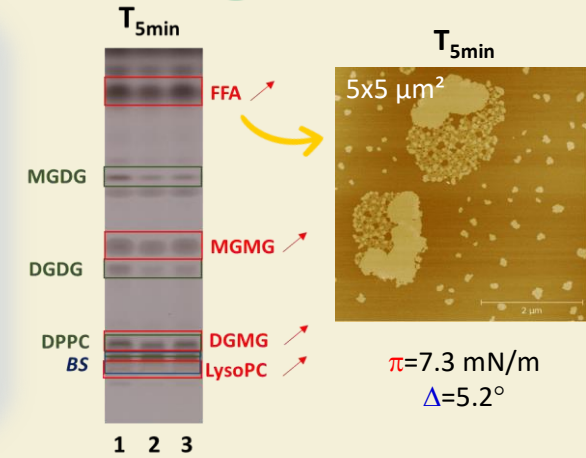


Results

Assembly scale (μ m)



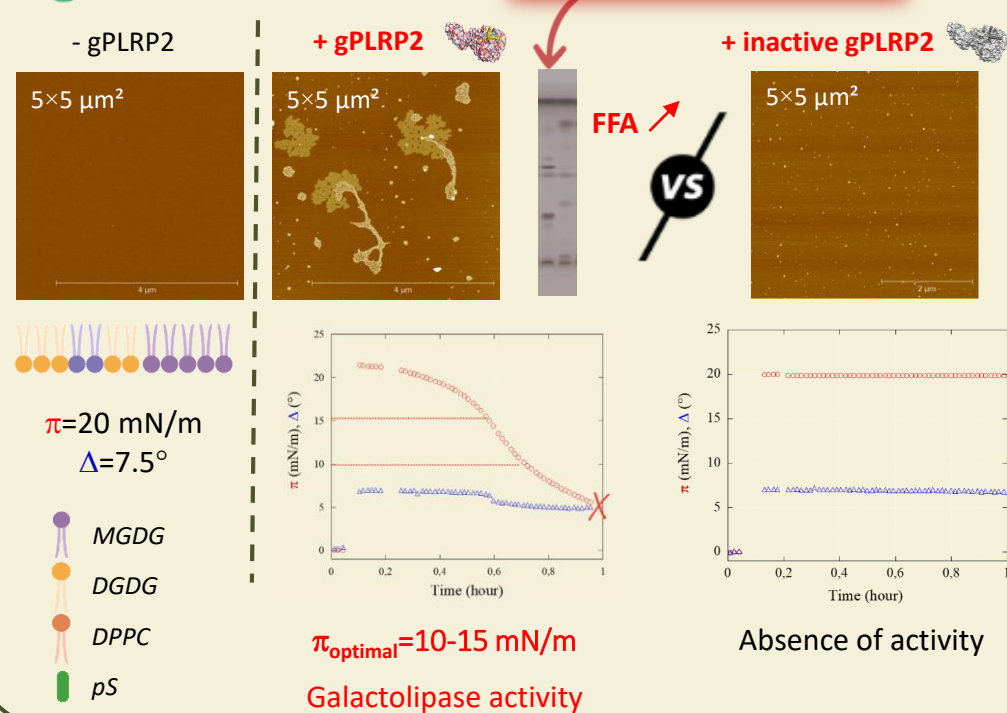
2 GL/DPPC/pS



Conclusion: In bulk, **no enzymatic activity** on GL-based liposomes in the absence of bile salts. In the presence of NaTDC (4 mM), gPLRP2 showed both **high galactolipase** and **moderate phospholipase A1** activities on liposomes, probably due to a decrease in packing and lateral pressure upon NaTDC adsorption, and subsequent disruption of liposomes.

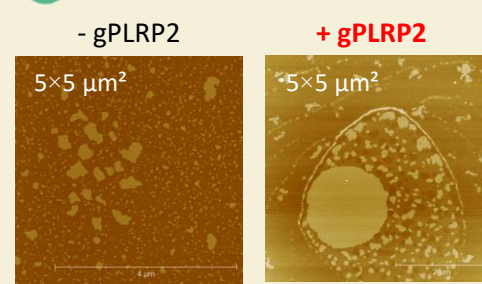
Results

1 GL

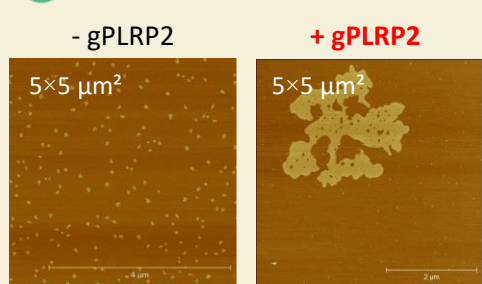


Enzymatic activity of gPLRP2
verified on GL monolayers

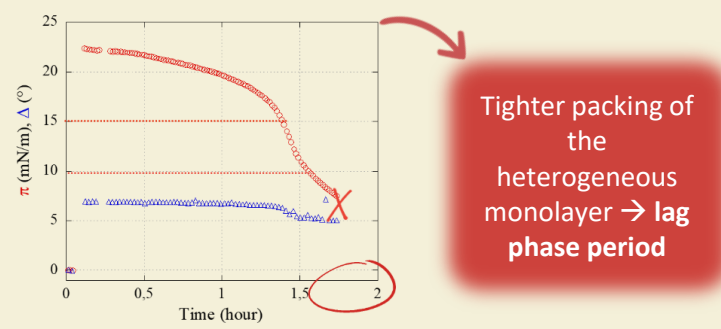
2 GL/DPPC/pS



3 MGDG/DGDG/SQDG/PG



Molecular scale (nm)



Conclusion: The **galactolipase** activity of gPLRP2 was evidenced at the level of **both homogenous and heterogeneous** GL-based monolayers. The presence of **charged lipids** (SQDG, PG) at the interface **improved the adsorption capacities** of the enzyme. The **optimal activity** of gPLRP2 was obtained in a range of surface pressure of **10-15 mN/m**.

Kergomard et al., 2021

Conclusions: Galactolipase and phospholipase A1 activities of gPLRP2 have been shown on **model GL-based monolayers**, but also on **liposomes** in presence of bile salts. It is now tempting to investigate whether gPLRP2 can act **directly on plant membranes**. Indeed, the galactolipase activity of PLRP2 at the level of natural membrane assemblies could open the way to the development of plant-based dietary alternatives to vectorize ω 3 PUFA, and contribute to the rebalancing of human and animal diets.

Infantes-Garcia et al., 2022
Mateos-Diaz et al., 2018

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