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What are the mechanisms of gastrointestinal lipases adsorption onto heterogenous biomimetic vegetal membranes?



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Objectives: to characterize main digestive lipases adsorption onto heterogeneous biomimetic vegetal membrane of increasing complexity and their lipolytic activities using complementary biophysical tools (tensiometry, ellipsometry, atomic force microscopy) and in vitro digestion.

Gastrointestinal lipases are crucial for lipid hydrolysis and must get adsorbed onto the substrate interface prior lipolysis. Such adsorption has not been deeply investigated on biomimetic vegetal membranes containing galactolipids as backbone. We had previously demonstrated on model milk fat membrane that phase **heterogeneity** modulated lipase adsorption. We wanted to check whether this behavior was generic and the nature of



Abbreviations: AFM, atomic force microscopy; rDGL, recombinant dog gastric lipase; DPPC,

Enzymes at work in Humans

Gastric lipolysis

pH 3 -5

Intestinal lipolysis

pH 6 -7.5

Polar lipid lipolysi:

Tensiometry/ellipsometry

Blodgett transfer

CONTROL – NO ENZYME



2.5x2.5 μm²

Dipalmitoylphosphatidylcholine; FFA, free fatty acid; GL, galactolipid; LE, liquid expanded; LC, liquid condensed; MGDG: monogalactosyldiglyceride; DGDG, digalactosyldiglyceride; rDGL, recombinant dog gastric lipase; lysoPC, lysopalmitoylphosphatidylcholine; pS, phytosterol; PLRP2, Pancreatic lipase related protein 2, PLA2, phospholipase A2

+ rDGL



- **rDGL** preferentially adsorbed onto the fluid phase and excluded from the condensed domains
- \checkmark In line with previous results on Milk bovine membrane Bourlieu et al., 2016; Bourlieu et al., 2019
- ✓ Phytosterols => better adsorption of rDGL on condensed phases and at edges of the LE/LC phase

Milk bovine membrane



✓ Optimal activity of gPLRP2 at 15 mN/m, galactolipase and phospholipase activity on GL/DPPC/pS monolayers and liposomes



Four height profiles

=2 nm (FFA, DPPC

enriched domain?)

to the background)

h=-0.3 nm (with respect

(MGDG, <mark>MGMG, DGDG</mark>,

DGMG in fluid phase?

h=0.5 nm (lysoPC?)

=4 nm (adsorbed enzyme?)



No lipolytic enzyme acting on polar lipids is observed in the gastric compartement

But the stability of emulsions resulting from gastric digestion influences the kinetics of intestinal digestion. Infantes-Garcia et al. 2021

Gastric lipase (HGL) – 47 kDa *Responsible for the digestion of 10 to 30% of TAG*

Highly active in acidic environment (pH 4-5.4)

Pancreatic lipase related protein 2 (PLRP2) – 50 kDa N'Goma et al. 2012



Responsible for the digestion of galactolipids

Andersson et al. 1995, Wattanakul et al. 2019

- *Low activity toward TAG and phospholipids* High galactolipase activity on galactolipid films and mixed
- *micelles (best susbtrate)*
- Specificity for the hydrolysis of ester bonds at the sn-1 position

* Pancreatic phospholipase A2 (PLA2) - 14 kDa Phospholipase activity on sn-2 position



Preference for anionic substrates (PG, PE)







 π =10.1 mN/m, Δ =6.4°

t (hour)





2.5x2.5 µm



Adsorption of sPLA2, more active at 10 mN/m than 20 mN/m, => FFA and lysoPC from DPPC, no activity on GL => self-organized domains enriched in DPPC or in FFA/lysoPC, activity on micelles

Adsorption of **PTL/coPTL** at the interface and subsequent disorganization of the lipid film, but no enzymatic activity, which is explained by its lack of activity on polar lipids

Conclusions - A strong surfactant property of GL and its preferential adsorption onto fluid lipid phase and at phase boundary were observed, in line with previous results on milk fat globule membrane. With PLRP2, changes in surface pressure indicated strong galactolipase activity and film reorganization. The injection of sPLA2-IB resulted in DPPC lipolysis only and changed the morphology of condensed domains. PTL/coPTL is adsorbed but has no activity. This study is a step forward to understand the interactions of gastrointestinal lipases with plant lipid membranes, an overlooked aspect of lipid digestion.

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