Label free Imaging of lipase activity on milk fat globules and model systems using DISCO beamline at Synchrotron SOLEIL

Bourlieu C. [1,3]*, Deglaire A. [1], Ménard O. [1], Pezennec S.[1], Le Feunteun S. [1,2], Villeneuve P.[3], Dupont D.[1], Fattaciolli J.[5], Jamme F.[6], Carrière F.[4], Vié V.[7]

[1] *INRA-Agrocampus Ouest UMR 1253 STLO, Rennes, France;*
[2] *UMR 782 INRA-AgroParisTech GMPA, Paris, France;*
[3] *CIRAD/INRA, UMR 1208 IATE, Montpellier, France;*
[4] *CNRS, Aix Marseille University, UMR 7282, Marseille, France;*
[5] *ENS UMPC UMR CNRS 8640, Paris, France ;*
[6] *Synchrotron SOLEIL, Saint-Aubin, France;*
[7] *Univ Rennes, CNRS, IPR - UMR 6251, F-35042 Rennes, France;*

*claire.bourlieu-lacanal@inra.fr

In their native state, milk lipids are present in the form of dispersed droplets called Milk Fat Globules (MFG, diameter 0.1-20 µm, average = 4 µm in bovine milk) [1,2]. The native MFG is constituted by a triglyceride core covered with an external trilayered membrane inherited from its secretory past. This membrane is mainly based on polar lipids (glycerophospholipids, sphingolipids and glycosphingolipids), proteins (25 % of total membrane with high proportion of glycoproteins and enzymes), neutral lipids and minor components. The major biological function of the MFG is to deliver energy to the mammal newborn, for that, the MFG has to be hydrolyzed in the gastro-intestinal tract by lipases, in successive steps. The lipases should interact with the MFG membrane and diffuse into the supramolecular object. The aim of this work was to determine the mechanisms involved in the enzymatic hydrolysis along the digestive process.
We used millifluidic cells designed to trap MFG or model droplets and injected successively each lipase (gastric, pancreatic related 2, pancreatic triglyceride lipase) involved in the digestion process and thus mimicking different physiological stages (preterm infant, fullterm infant and adult). Thanks to the deep UV-microscope present at DISCO beamline, the autofluorescence of tryptophan (and tyrosine) of lipase amino-acids allowed protein observation without external labeling.
The gastric and intestinal neonatal disintegration of milk fat globules (human or bovine) or other model systems (bovine anhydrous milk fat droplets stabilized by bovine milk membrane extracts or soy lecithin) was approached through transmission images recording. The analysis of the number of globules and their state of aggregation/coalescence during digestion (approached via diameter, size, mode and specific surface evolution against time) gave indication on the kinetics and mechanisms of lipolysis. Fluorescence (327-335 nm) image indicated lipase distribution.
Gastric phase was mainly marked by the heterogeneous adsorption of the gastric lipase in the MFG membrane. Afterwards, heterogeneous adsorption of pancreatic triglyceride lipase could also be observed followed by the disappearance of the MFG core. The progression of lipases fronts was monitored and the kinetics of lipolysis characterized allowing the differentiation of substrate and of *in vitro* digestion models (full term infant, versus preterm infant versus adult). The microfluidics set up was a very interesting tool to follow sequentially the action of lipases, to save volume of purified lipase and to image simultaneously several positions. The high sensitivity of the DISCO beamline allowing the detection of digestive lipases auto-fluorescence, is a unique tool to unravel their mechanisms of adsorption onto complex substrate using free-label imaging and

has generated useful data for infant formula optimization.

[1] C. Bourlieu, S. Bouhallab, and C. Lopez. Biocatalyzed modifications of milk lipids: applications and potentialities, Trends in Food Science & Technology, 20 (2009) 458-469.
[2] C. Bourlieu, and M-C. Michalski. Structure–function relationship of the milk fat globule. Current Opinion in Clinical Nutrition and Metabolic Care 18[2], 118-127. 2015.