Yeast lipid bodies under sunlights, dynamic and structural studies using SMIS and DISCO beamlines

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Lipids body: a complex and dynamic organelle

In cells, neutral lipids (triglycerides and sterol esters) are stored in organelles called lipid bodies (LB) [1]. They are present in all organisms, from bacteria to plants and animals.

Lipid bodies: not well known but with rising interest

From biologists
- LB is not an inert fat depot but a dynamic organelle which regulates cell metabolism and signaling

From medical field
- LBs have a crucial role in diseases with increasing prevalence (obesity, diabetes) [2]
- Oleosins (from peanut and hazelnut)’ seed LB associated proteins are allergens [3]

From industrial
- crushing - oils for food and non food (biopharmaceuticals; productions) are extracted from seed LB
- food processing industry, cosmetic and health - oleosin harbor interfacial properties and could be use as emulsifying agents or in drug delivery systems [4]

Lipid bodies are targeted to lipid bodies in S. cerevisiae

Photic microscopy pictures (bright field and epifluorescence) of yeast expressing Erg6p-RFP (lipid body) and AtS3-GFP (lipid body-associated protein) [5]

Oleosins, seed lipid body associated proteins

Oleosins are LB integral proteins
- Predicted structure: trimeric organization
- variable N-terminal and C-terminal part, exposed at the surface
- highly hydrophobic central part inserted into the phospholipid monolayer and/or the TMS core.

Oleosines are targeted to lipid bodies in S. cerevisiae

Photonic microscopy pictures (bright field and epifluorescence) of yeast expressing Erg6p-RFP (lipid body) and AtS3-GFP (lipid body-associated protein) [5]

Neutral lipid content heterogeneity revealed by single cell FTIR analysis

Cells are on-dried on ZnSe hemisphere
- Spatial resolution: 4x4 µm
- Single cell spectrum acquisition
- Fluorescence acquisition

Neutral lipid increase is correlated with global metabolic modifications

Metabolic modifications were detected using FTIR and GPC by biochemical analysis
- Using ZnSe hemisphere and spectrophotometry, we obtained yeast single cell FTIR spectra. We confirmed the cell heterogeneity observed with fluorescence microscopy.
- We observed that lipid accumulation induced global metabolic modifications. These results were confirmed by biochemical analysis and revealed a link between storage lipid and storage carbohydrate fluxes.

RESULTS

STRUCTURAL STUDIES

AtS3 oleosin structures in surfactants are contradictory

Using SRCD on a DICO beamline, we obtained the structure of AtS3 oleosin solubilized in various surfactants, AT, SDS, Alkapol 0.5% A-35K and 10 mM Foscholine [6]

AtS3 oleosin is massively associated with lipid bodies in yeast

Purified Lb are analyzed:
- using microscopy
- using dynamic light scattering
- The associated proteome are analyzed by SDS PAGE

AtS3 oleosin structure in natural environment is mainly beta

We obtained SRCD data on whole organelle and information on the AtS3 oleosin in a natural environment, lipid bodies. We observed that the fold in LB was the same as the fold of AtS3 solubilized in Poschilane.

Conclusion and perspectives of structural studies
- We obtained the first SRCD data on whole organelle and information on the AtS3 oleosin in a natural environment: lipid bodies. We observed that the fold in LB was the same as the fold of AtS3 solubilized in Poschilane.
- We would validate our results using SRCD-FTIR coupled analysis on dry filers. We obtained the first spectra on DICO and SMIS in December 2021.

DYNAMIC STUDIES

Neutral lipid increase is correlated with global metabolic modifications

Metabolic modifications were detected using FTIR and GPC by biochemical analysis
- Using ZnSe hemisphere and spectrophotometry, we obtained yeast single cell FTIR spectra. We confirmed the cell heterogeneity observed with fluorescence microscopy.
- We observed that lipid accumulation induced global metabolic modifications. These results were confirmed by biochemical analysis and revealed a link between storage lipid and storage carbohydrate fluxes.

Conclusion and perspectives of dynamic studies
- We will determine if these metabolic modifications are the consequence of pathway regulations at the transcription level by conducting a transcriptomic analysis of yeast cells.