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SOLEIL sheds the light on lipid bodies protein structure

SCIENTISTS INVOLVED

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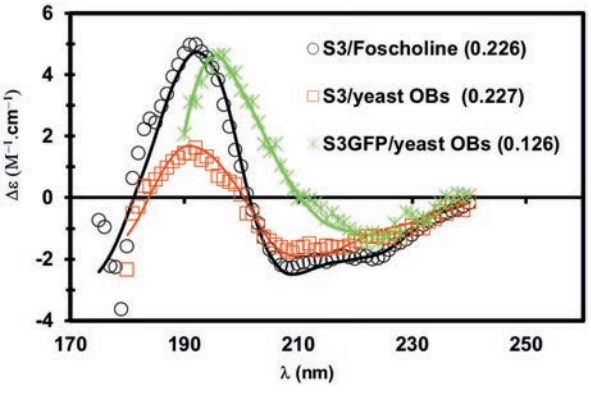
KEYWORDS

Lipid bodies, oleosome, Synchrotron Radiation Circular Dichroism, Small Angle X ray Scattering, low soluble proteins, protein interaction, lipid design, Arabidopsis thaliana, Saccharomyces cerevisiae.

SCIENTIFIC QUESTION

Most of the organisms studied to date accumulate storage lipids in specialized structures called oil bodies (OB). These intracellular inclusions have a diameter close to the µm, and their organization is unique among organelles. They consist in a core of neutral lipids (triglycerides, sterol esters) surrounded by a monolayer of phospholipids (PLs) in which various proteins are found. While oil bodies have been considered during almost a century as inert balls of fats, they recently earned the status of real organelles, with they own dynamics and original structure. The major questions on OB from various organisms (from crop to humans) are how lipids are accumulated, stabilized and at last mobilized. The nature of the proteins associated with OB, their structure, and their insertion in a unique monolayer are still opened questions. Oleosins are the most abundant proteins of seed OBs. They belong to a multigenic family, with a unique triblock structure (a hydrophobic core flanked with two amphiphilic regions). The N and C terminal regions of oleosins show considerable variation in term of size and sequence. Their hydrophobic segment, the longest known to date, is inserted in a PL monolayer specific of the organelle. Although Oleosins role in organelle stabilization and in the lipids mobilization during the germination [1] is known, data about their secondary structure are scarce and contradictory [2].

The secondary structure of surfactant or organic solvent solubilized oleosins has been investigated by different authors using Fourrier-Transformed Infra-Red microscopy (FT-IR) and Circular Dichroïsm (CD). Oleosins in purified OBs where also studied by FT-IR [2]. All these studies, in very different environments, gave contradictory results on oleosins secondary structure (either maintly α-helical or β-strand folded).



▶ *Figure 1: CD Spectra of oleosin.*

Approaches aimed at maintaining unique oleosin isoforms soluble, used amphiphilic polymers (Amphipols), conventional detergents, or eukaryotic expression system targeting unique oleosins isoforms to OBs with diameter suitable for spectroscopic methods. Synchrotron Radiation CD (SR-CD) at DISCO beamline permits to record spectra on samples very diluted, or diffusing UV light, and at low UV wavelength (180 nm), thus permitting a fine characterization of the β content of proteins [3]. SAXS at SWING beamline also allowed characterizing the protein amphipol assemblies.

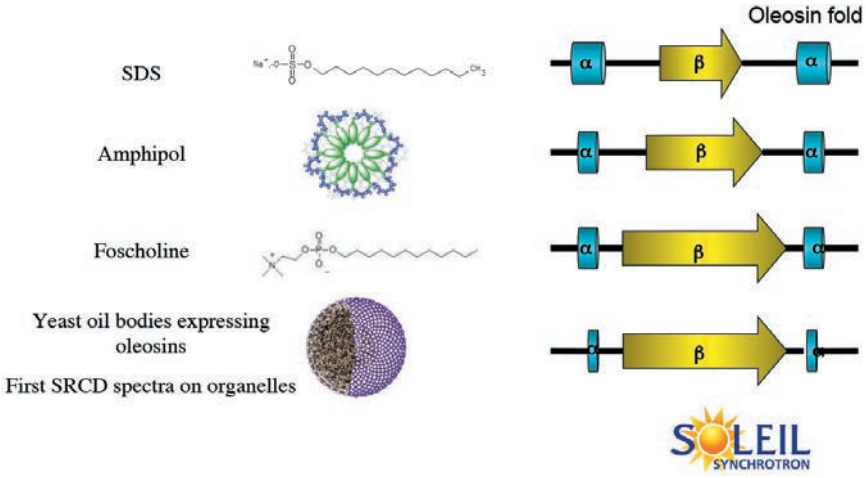
▸ MAIN RESULTS AND PERSPECTIVES

Fold of oleosin solubilized by various surfactants

Different oleosins isoforms were maintained soluble in aqueous solution, using various detergents or original amphiphilic polymers, amphipols. Oleosins, insoluble in water based buffers, were maintained soluble (10 min at 200 000 xg) either by anionic detergents or amphipols. Neutral detergents were ineffective. Comparing hydrodynamic radius (obtained by dynamic light scattering) to radius of gyration (obtained by Small Angle X-Ray Scattering, SWING beamline) permitted the characterization of the sphericity of various Apols wrapping around oleosins. Size and dispersity of the complexes were sufficiently low to permit SR-CD measurements. SR-CD spectra were successfully recorded for all complexes. Analysis of these data proved that in all media, oleosins were folded. When maintained soluble with amphipols the oleosins possess more beta and less alpha secondary structures than in the SDS detergent (a denaturing molecule). These are the first reported structural results on lipid bodies proteins maintained in solution with amphipols, a promising alternative to notoriously denaturing detergents.

Fold of oleosin in an oil body environment

To study the fold of integral membrane proteins inserted in a cellular OB environment, the major *Arabidopsis thaliana* seed oleosin (S3), was targeted to *Saccharomyces cerevisiae* OBs. The diameter of purified yeast OBs harboring S3 or S3 fused with the Green Fluorescent Protein (GFP) was smaller and more homogeneous than plant OBs. Comparison of the secondary structure of S3 and S3–GFP was used to validate the structure of folded S3. SR-CD measurements indicated that S3 and S3–GFP in yeast OBs contain mainly beta secondary structures. While yeast OBs are chemically different to *A. thaliana* seed OBs, this approach allowed the secondary structure of S3 oleosin in OB particles to be determined for the first time. These results were consistent with the high beta content of the hydrophobic central domain of oleosins observed in amphipols polymers and in foscholine is an analogue of phospholipids, thus validating both approaches.



▸ *Figure 2: Model of the fold of oleosins in various environments. In blue, the hydrophilic extremities; in yellow, the hydrophobic central part. At the bottom, the folding models proposed with beta-sheets in the form of arrows and alpha helixes in the form of cylinders.*

▸ CONCLUSION

The use of various biophysical methods (Synchrotron Radiation Circular Dichroism, Xray scattering, available at SOLEIL Synchrotron) permitted to characterize oleosin fold in both environments: surfactants or native-like yeast OBs. These approaches lead to a secondary structure model for oleosins in which the central hydrophobic region adopts an original beta fold among eukaryotic proteins [4, 5] (Fig. 2).