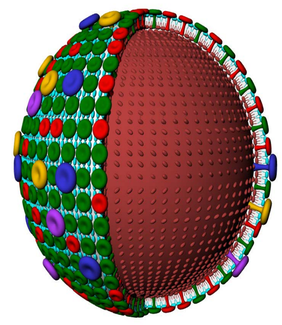
SOPOL SOlubilisation des Protéines de l'OLéosome (Oil body protein solubilization for structural studies)

CP2D 2008

INRA, AgroParisTech, UMR1318 IJPB, Versailles, P Briozzo, T Chardot, M Froissard, Y Gohon, F Jagic, P Jolivet, M Miquel, Z Purkrtova, R Tache, J-D Vindigni; Ecole Normale Supérieure UMR8640 CNRS ENS UPMC Paris, E Marie, A Pallier*, R Liu*, C Tribet; SOLEIL Synchrotron, DISCO Beamline, Saint Aubin, A Giuliani, F Wien; UMR INRA CIRAD, AGAP Montpellier, S Catrix, F de Lamotte

Aim of the project

Seeds store reserve lipids in subcellular organelles called oil bodies (OBs) until germination. OBs constitute an oil-in-water natural emulsion, covered by a phospholipid monolayer and stabilized by tri block hydrophobic proteins, oleosins and caleosin, for which no reliable structure is known. The difficulty in extracting oil from numerous seeds is reflected by the complexity of the processes used to this effect (high energy and harmful solvents i.e. hexane). Moreover, these processes strongly affect the quality of protein cakes, used in animal feed. Knowledge of OBs structure and stability are prerequisites for designing extraction schemes using conditions as mild as possible. Using various biological and physical approaches we wish to structurally characterize "structural proteins" from seed OBs and give a molecular basis to OBs stability.

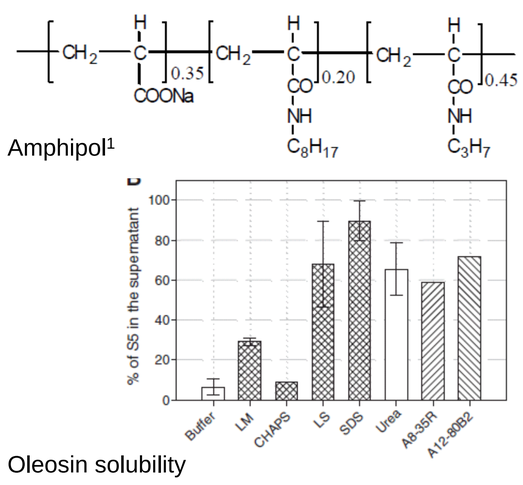


Model of OB structure. Hydrophobic core (brown), phospholipid monolayer (pale blue) and proteins (colored mushrooms)

Oleosins are maintained soluble and folded using various amphiphilic polymers.

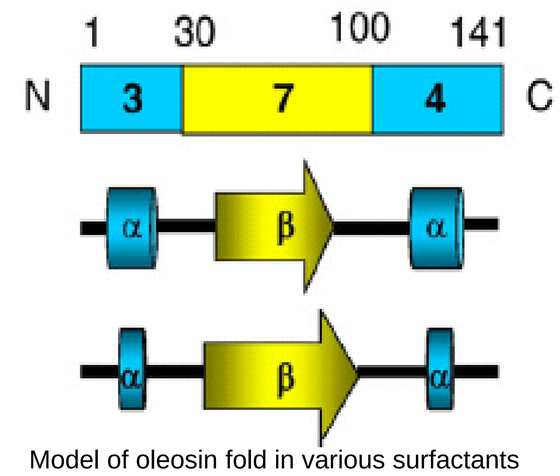
We have used various molecules and biophysical approaches to maintain oleosins soluble and to study their fold.

Among surfactants used in this study, only anionic detergents (lauryl sarcosine, SDS) and amphipols[1] (blocky A12-80B2, random A8-35R), maintained oleosins soluble after ultra centrifugation at 200.000 x *g*



Oleosin solubility

Synchrotron Radiation Circular Dichroism (SRCD) experiments indicated that oleosin surfactant complexes are folded. Oleosin central region exhibits an original fold (β-sheet)[1, 2].



Model of oleosin fold in various surfactants

Oleosins are folded in natural oil bodies.

Oleosins are not correctly folded in reconstituted oil bodies[2-4], but SRCD experiments showed that purified seed OBs contain folded proteins. S3 oleosin has been maintained soluble and folded in foscholine, a detergent with headgroup analogue to a phospholipid, thus mimicking oleosin natural environment. Upon expression in yeast, S3 oleosin is targeted to oil bodies, and is the most abundant protein. SRCD results confirm the tendency of oleosins to adopt an original structure with a majority of β–sheet folds[4].

S3 environment	Alpha	Beta	Turns	Unordered and/or not assigned
SDS*	41	17	11	31
APol*	27	25	13	35
Foscholine 12	18	33	12	37
Yeast OBs	13	33	15	39

Secondary structure of S3 oleosin in various environments determined by SRCD

Conclusion

It is the first time that, using rigorous criteria, OB proteins are maintained soluble in aqueous media. We proved that protein surfactant complexes exhibit low polydispersity and are folded. We propose that the central region of oleosin adopts a original β–sheet fold.

Publications

1) Popot, J.L., et al (2011). Amphipols from A to Z. Annu Rev Biophys 40, 379-408.
Gohon Y. *et al*. (2011) High water solubility and fold in amphipols of proteins with large hydrophobic regions: Oleosins and caleosin from seed lipid bodies. Biochim Biophys Acta 1808: 706-716.
2) Vindigni J.D. (2011) Solubilisation des oléosines de graines d'*A. thaliana,* études structurales pour la valorisation. Thèse de doctorat AgroParisTech.
3) Vermachova M. *et al.* (2009) Surface mapping of plant oil-bodies' proteins. FEBS J. 276, 170-171 S 1
4) Vindigni J.D. *et al.* Submitted

* ESPCI UMR 7615, Paris

CONTACT :
Thierry Chardot, INRA AgroParisTech, IJPB Versailles
http://www-ijpb.versailles.inra.fr/en/bs/equipes/biostructurale/index.htm
thierry.chardot@versailles.inra.fr