SRCD-FTIR coupled study of hydrophobic integral protein fold in lipid bodies

Marine Froissard

Standard Project

Experimental Report

Proposal title: SRCD-FTIR coupled study of hydrophobic integral protein fold in lipid bodies		Proposal number: 20110107
Beamline: DISCO and SMIS	**Date(s) of experiment:** from: 14 of October to: 16 of December	**Date of report:** 31 of march
Shifts: 9 + 12	**Local contact(s): Alexandre GIULIANI and Frédéric JAMME**	*Date of submission: 31 of march*

Objective & expected results (less than 10 lines):

Highly hydrophobic proteins, including integral membrane proteins are of major interest for the scientific community due to their economical and biotechnological importance. Among them, a particular class is associated with the phospholipids monolayer of lipid bodies (LBs). These proteins share structural properties and are also connected to agronomic stakes (oleosins) or health issues (apolipoproteins and PAT proteins). Thanks to an heterologous expression system we developed in yeast, we are able to produce purified lipid bodies harboring these proteins and suitable for structural analysis. We will use this powerful tool to study the fold of hydrophobic integral protein in lipid bodies using SRCD-FTIR coupled approaches. This study will confirm our first results obtained in 2010 on AtS3 oleosin.

Results and the conclusions of the study (main part):

Production of lipid bodies harboring integral membrane proteins

We obtained 6 types of samples, lipid bodies harboring, no heterologous proteins, plant proteins (AtS3-GFP, AtS3, AtS5 or AtClo1) and mammalian protein (bovine stomatine BtStom) Surprisingly, for the Hepatitis C virus core protein, we did not obtain LB association of the protein, in contrast with the results obtained after its expression in mammalian cells

After biochemical analysis, we concluded that only samples with AtS3 isoforms were suitable for structural analysis, with a high content of heterologous protein associated with purified LBs (Figure 1). We modified our experimental procedure (culture conditions) for the other proteins but we did not obtain a better heterologous protein association with LBs (Figure 1)

Part 1: sFTIRmicrospectroscopy on purified LBs (SMIS beamline)

The main challenge was to obtain samples suitable for sFTIR spectra acquisition (dry and without salts). It was not possible to maintain LBs in water (aggregation of LBs) so we used LBs in Tris NaF buffer (salt conditions for SRCD experiments). Drops of few microliters were successively dried on ZnSe ATR hemisphere and washed with water to obtain LB clusters (5x5 µm) convenient for analysis with the high resolution Synchrotron FTIR microspectroscopy with the Continuum XL microscope. We carefully scan the hemisphere to select a region for spectra acquisition on **native LB clusters**. Individual spectra were saved in log(1/R) format at 8 cm^{-1} spectral resolution, with 512 co-added scans encompassing the mid-IR region from 4000 to 800 cm^{-1} to increase the signal to noise ratio. We obtained 20 spectra per sample (LBs with no heterologous protein, LBs with AtS3 or AtS3-GFP) in these conditions.

Part 2 : SRCD-FTIR coupled analysis on purified LBs (DISCO and SMIS beamlines)

To compare results obtained with CD and IR, we developed sample preparation on CaF2 slides allowing its analysis consecutively by these two methods without any treatment between CD and IR spectra acquisitions. LBs in Tris NaF buffer were first analyzed by SRCD in solution. The same LB sample was then air dried on CaF2 slide and CD spectra were also acquired. The slide was transferred on SMIS beamline for IR transmission analysis on **large LB clusters** (50x50 µM) with the Nicolet iN 10 (not with high resolution

synchrotron FTIR microspectroscopy). **We recorded for a same CaF_2 slide containing LB sample, CD and IR spectra** (Figure 2). For LBs harboring AtS3 protein, SRCD measurements on dried films (not shown) unfortunately didn't give comparable signal to the one in solution. But, for S3-GFP, similar signal was observed in solution or on dried films. These results indicate 1) film preparation and drying procedure are critical steps 2) similar secondary structure in solution or on dried films can be observed. Moreover, secondary structure analysis and comparison between FTIR and SRCD approaches didn't give similar results. Nevertheless, tendency is the same. Meaning that with both techniques S3-GFP proteins have more beta sheet content than S3. This is consistent with the beta fold of GFP protein (52% of beta, 20 % of alpha and 16 % of turn structures).

Conclusion and perspectives

Using SRCD and high and low resolution FTIR, we obtained a large set of data on purified LB harboring plant protein. We are now performing fine computational analysis of the results to compare structural information obtained with these two approaches.

Figure 1: protein profile of LBs harboring heterologous proteins.

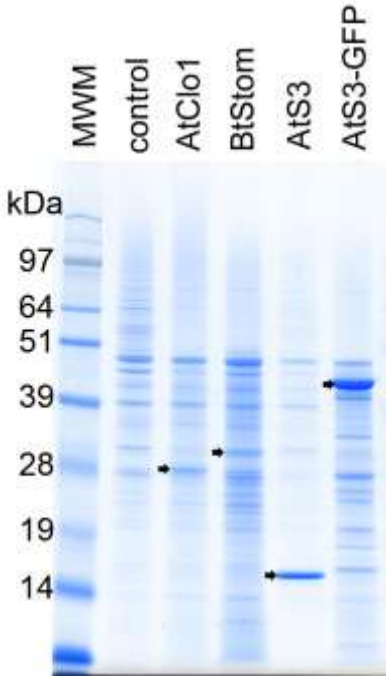
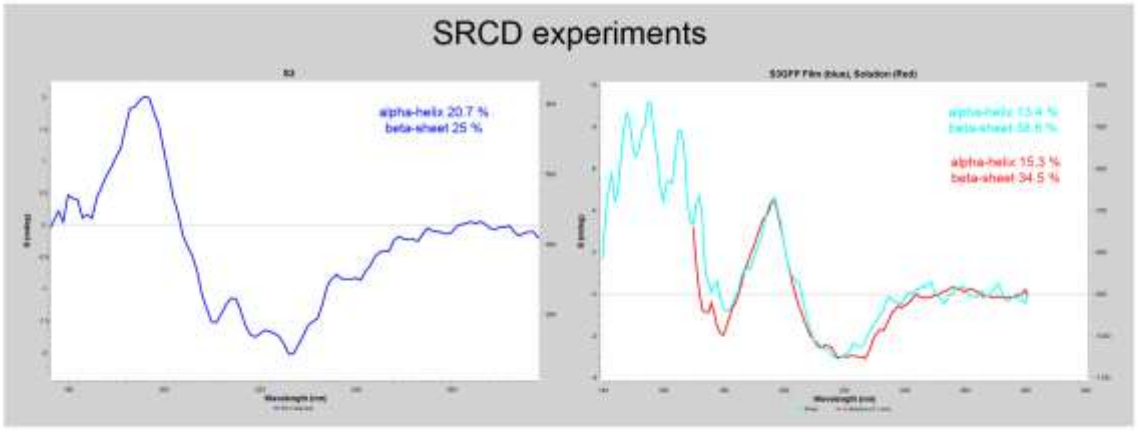
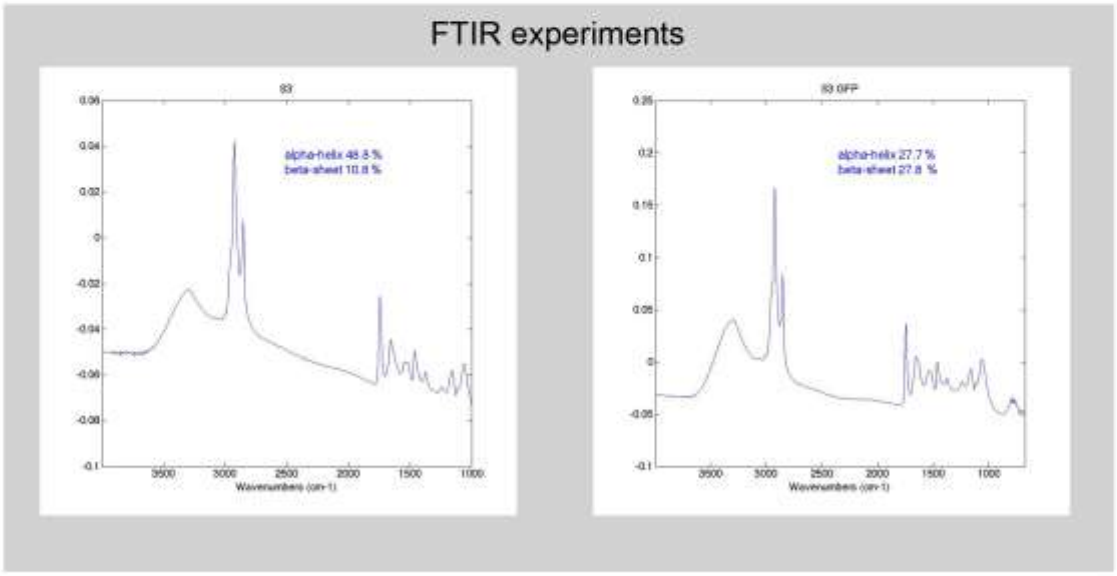


Figure 2: SRCD and IR spectra of LBs harboring AtS3 or AtS3-GFP





Justification and comments about the use of beam time (5 lines max.):

The beam time of 12 shifts, allocated for the sFTIR analysis of the present proposal, corresponded to the realization of the depicted experiments. We effectively needed this time, to prepare samples, to find good conditions to maintain native lipid bodies after drying and desalting on ATR hemisphere, to make zone selection and microscope alignment and to acquire spectra with a low signal to noise ratio (512 co-added scans).
The beam time of 9 shifts, allocated for the SRCD analysis of the present proposal, corresponded to the realization of the depicted experiments as we developed various procedures to analyze LB samples in Tris NaF buffer or dried on CaF2 slides compatible with IR coupled study.

Publication(s):
Work described above to be submitted.