Structuring membrane proteins as tools in material science

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ABSTRACT

Integral membrane proteins are interesting from a material science point of view due to their wide variety of functions and the possibility to create a functional barrier or carrier system. By using the same molecules found in nature, the structure and properties can be mimicked and utilized in designed materials.

Two membrane proteins derived from yeast, Yop1p and Rtn1p, have been engineered, heterogeneously expressed and purified using a GFP-fusion platform. Both proteins are membrane spanning and can be found in all eukaryotes in which they are used for structural control of the endoplasmatic network. Yop1p and Rtn1p have previously been shown to induce tubules in lipid vesicles and we have seen that by altering the concentration of structuring protein as well as the shape of the lipids, the vesicle morphology can be controlled.

Keywords: membrane proteins, self assembly, curvature, cryo-TEM, biomimetic

1 INTRODUCTION

Integral membrane proteins comprise 25% of the proteins encoded by our genome and have a wide variety of functions such as transport, signaling, adhesion and structuring of the membrane. What makes membrane proteins interesting from a material science point of view is their usefulness as catalysts, channels, pores or structuring agents whilst immobilized in an enclosed molecular entity such as a vesicle or a combination of an inorganic solid with a surrounding soft matter [1, 2].

Yop1p or DP1 is a membrane protein found in all eukaryotes. It is involved in shaping the tubular network of the endoplasmatic reticulum, ER [3]. In cells, the ER is usually arranged as a network of branched tubes but shapes as stacked arrays, lamellae, karmellae or highly ordered arrays with cubic symmetry [4] has also been seen. Previously, Yop1p has been shown to induce tubes in lipids. It is believed that Yop1p can induce this structural change by inserting two hydrophobic domains that can form a hairpin or wedge in the membrane. It was also found that Yop1p can polymerize into short tubular rings, something that likely enhances the structural rearranging efficiency [5]. Rt1p is a protein from the reticulon family and is also involved in shaping the ER [6]. The protein likely acts in a similar way with a wedge shaped hydrophobic part but also consists of a larger cytosolic domain.

The functionalization of materials using membrane proteins can be divided in three parts. The first step involves purifying proteins from the source. This step is aided by adding an affinity tag (His) and a fluorescent tag (GFP) to the protein. By combining these two a high throughput screening can be done followed by fluorescent size exclusion chromatography (FSEC). The second step is reconstitution in to a matrix of choice, e.g. vesicles. A third optional step includes a further functionalization by using the lipid/protein matrix together with inorganic carrier, thus creating a hybrid material.

In this study we have used a GFP-fusion based technology to purify eukaryotic membrane proteins and induce tubular structures in a lipid matrix. It has been shown that the proteoliposome morphology depends on the structure and phase of the lipids.

2 METHODS

A method to engineer, express and purify membrane protein have been used, similar to [7]. In short, the protein of choice is amplified using suitable primers from wild-type *S. Cervisiae* DNA and cloned into a *SmaI* linearilized vector with a yEGFP, octa-His tag and a TEV protease site at the C-terminus. For expression, the vector and the PCR product are transformed into the *S. Cervisiae* strain FGY217 (MATa, *ura3*-52, *lys2*Δ201, *pep4*Δ). The over expression levels can, due to the GFP-tag, easily be screened by measuring whole-cell fluorescence and membrane suspension fluorescence in a microplate spectrofluorometer, SpectraMax M2e (Molecular Devices) and also by running a SDS-PAGE and detecting in-gel fluorescence with a LAS-1000-3000 CCD imaging system (Fujifilm).

The yeast strain containing the membrane protein construct is grown in –URA media and expressed using galactose for 23 hours. The membrane suspension is, after centrifugation and cell breakage, solubilised in a detergent of choice at a concentration of ~2 times CMC. A HPLC-based high throughput detergent screen is performed in order to find the most suitable detergent for a specific protein. The solubilized proteins are purified using the His-tag on a His-Trap column (GE Healthcare). The eluate is

loaded on a size separating column, Superdex-200 (GE Healthcare). The GFP and His-tag is cleaved off by incubating the sample in His-tagged TEV protease over night and running the whole sample through a His-Trap column.

The lipid matrix is prepared by dissolving the lipids in buffer and an initial freeze-thawing in liquid nitrogen for five times to get multilamellar vesicles. This is followed by either extrusion through polycarbonate filters with a pore size of 100 nm for ten times or by ultrasonication for 10 minutes, both in order to create unilamellar vesicels with a size ~150 nm, verified by dynamic light scattering.

To change the environment of the membrane protein, from the detergent to the material of choice, several methods can be used. Biobeads SM-2 (BioRad) removes the detergent using polar interactions and can be added to a solution of detergent/protein and preformed vesicles. Other methods include dilution or dialysis.

The lipid/protein complexes are studied with Cryo-transmission electron microscopy using a Zeiss 902 A instrument, operating at 80 kV. The specimens are prepared by blotting a very small drop of sample in a chamber with high humidity onto a copper grid coated with a polymer film. The film is vitrified by a rapid plunging of the grid in to liquid ethane. A cryo-holder maintains the low temperature during transfer and the sample is imaged at 100 K.

3 RESULTS AND DISCUSSION

A combination of affinity chromatography and GFP-fusion technology improves the success in obtaining pure and correctly folded membrane protein. The GFP moiety is attached to the C-terminal of the protein and since it folds correctly and becomes fluorescent only if the protein is correctly integrated in the membrane it can be used as an indicator of correctly folded proteins as well as a probe. Fluorescence can quickly be measured in liquid cultures, SDS-gels and detergent solubilized membranes and is much more sensitive than other staining methods or UV/vis-spectroscopy.

To solve the problem of aggregation, detergents are used in order to solubilize the protein. A multitude of detergents suitable for protein solubilization are available and in order to select the best parameters, a method based on FSEC and a HPLC system that can screen a multitude of detergents simultaneously, both after solubilization and after IMAC purification, have been used in this study. The method requires very small sample volumes and allows a quick screening of several proteins by determining the monodispersity of the spectrogram. This give a good overview of how a protein acts in different detergents and, if necessary, helps in finding a detergent with specific properties, e.g. surface charge or CMC. An example of such a screening can be seen in figure 1.

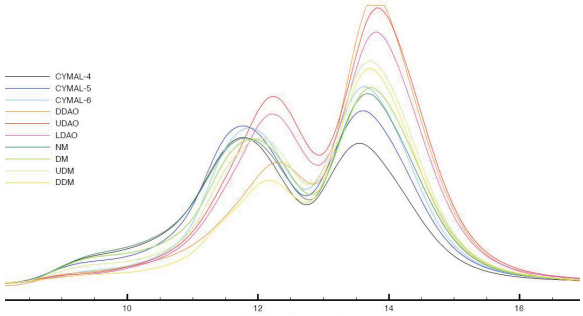

Figure 1. Spectrogram showing the solubilization effect of different detergent on Rtn1p. By using the fluorescent tag on the proteins and a high throughput HPLC system many detergents can be screened simultaneously, requiring very small sample volumes. Relative fluorescence is plotted against retention time in minutes.

Yop1p is predicted to consist of four tilted α-helices with two oppositely charged amino acids just below the lipid headgroup. This configuration makes the protein wedge shaped which is, together with its ability to polymerize, one of the reasons why it shapes membranes (fig. 2).

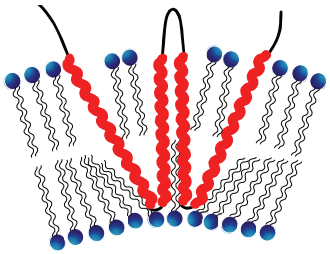

Figure 2. Schematic diagram of Yop1p in a lipid bilayer. Yop1p contain four membrane spanning motifs. The ability of Yop1p to form tubes can possibly be derived from two oppositely charged amino. This makes the helices bend and assume a hairpin formation.

1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) is a naturally occurring phospholipid with a cylindrical shape. Such a lipid easily forms vesicles due to its shape and propensity for flat surfaces (fig. 3). This effectively means that the propensity for tubes is very low due to its high curvature.

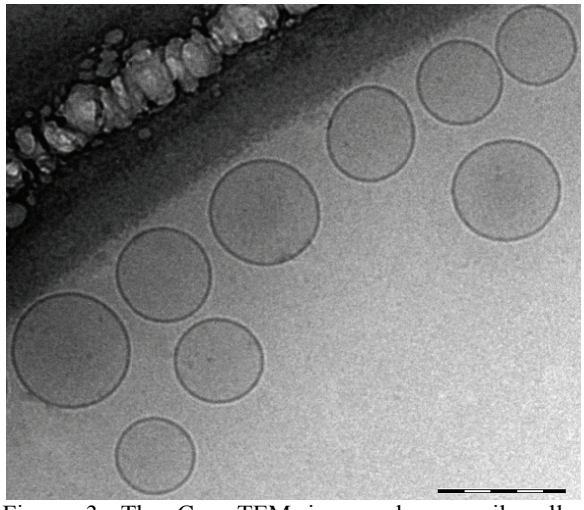

Figure 3. The Cryo-TEM image shows unilamellar vesicles of DOPC after extrusion. Scale bar 200 nm.

When Yop1p is reconstituted into vesicles of DOPC proteoliposomes shaped as tubes are formed, see figure 4, already at such low concentrations as 5 wt% but also at such high concentrations as 50 wt%. When Yop1p is added to a lipid/detergent solution, without any preformed structures, tubes were formed as well, many μm long and ~50 nm in diameter (fig. 5).

The tubes seen in figure 3 have a relatively high mean curvature. The Yop1p is reconstituted in a virtually flat surface and most likely curves this by bending the outer leaflet of the membrane. Certain vesicles contain fewer proteins which results in protruding tubes from an otherwise spherical vesicle, as can be seen in figure 6.

By varying the lipid composition and morphology and the protein concentration relatively flat DOPC membranes can be induced to form rod shapes due to an overall shift from unity of the overall molecular packing parameter, S [8].

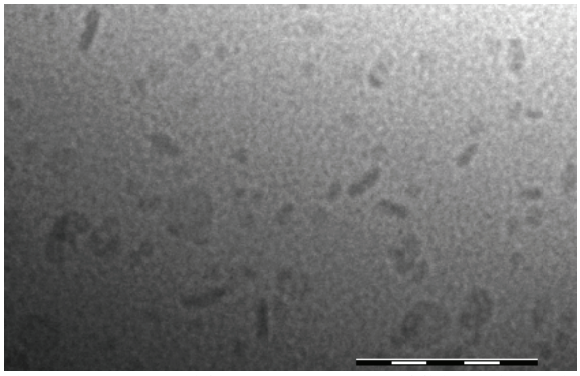

Figure 4. DOPC with 5 wt% Yop1p. Addition of protein to preformed vesicles of ~100 nm gives small 15 nm wide tubes. Scale bar 200 nm.

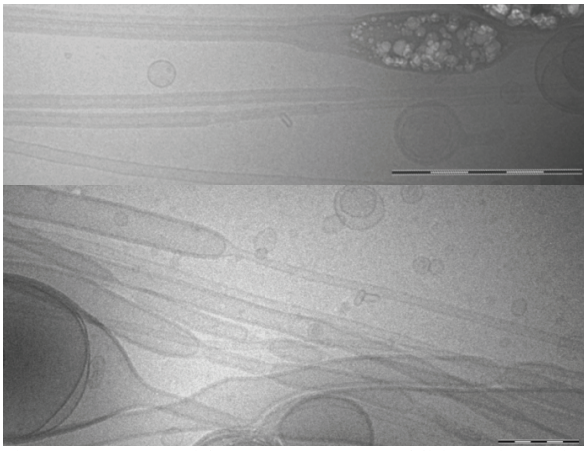

Figure 5. DOPC with 5 wt% Yop1p. Addition of protein to disordered lipids gives tubes in the μm length scale. Scale bar 500 nm (top) and 200 nm (bottom)

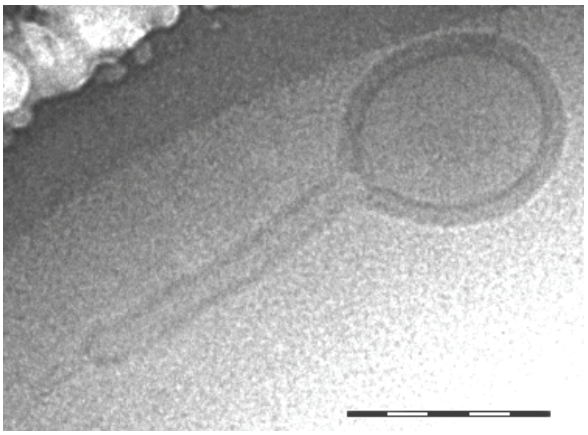

Figure 6. Addition of Yop1p to vesicles of DOPC. A vesicle with a single protruding tube. The vesicular part consists of four membranes, the long extension of two and the short of a single membrane. Scale bar 200 nm.

4. CONCLUSIONS

In this paper we have utilized a high through put platform for heterogeneous expression of eukaryotic membrane proteins for biomimetic materials design based on affinity chromatography and a fluorescent tag. Yop1p and Rtn1p are two membrane proteins expressed and purified. Both are eukaryotic membrane spanning proteins involved in the shaping of the ER membrane. We have shown by cryo-TEM that reconstituting Yo1p in lipids without any tendency for curvature give tubes with relatively large mean curvature.

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