Combining detergent screening and size homogeneity analysis of histidine-tagged membrane protein using His MultiTrap FF and Superdex 200 5/150 GL

Application

Purification of membrane proteins requires solubilization of the membranes using mild detergents, and separation in the presence of detergent to keep the proteins in solution. A suitable detergent should be capable of extracting the target protein and should allow purification without loss of native structure and function of the protein. A stable and homogenous preparation of the target protein in detergent solution is key to success in structural studies by crystallography or NMR.

Multiple detergents need to be tested to identify the detergent giving best yield, purity, and size-homogeneity of the membrane protein. This work often needs to be performed with very small amounts of protein since membrane proteins often express at low levels.

This Procedure describes the steps for screening for optimal detergent type and concentration, target protein enrichment on His MultiTrap™ FF 96-well plate, and size homogeneity analysis[1] on Superdex™ 200 5/150 column.

1 Size homogeneity is a useful indicator of stability as membrane proteins tend to oligomerize or aggregate rapidly when destabilized.

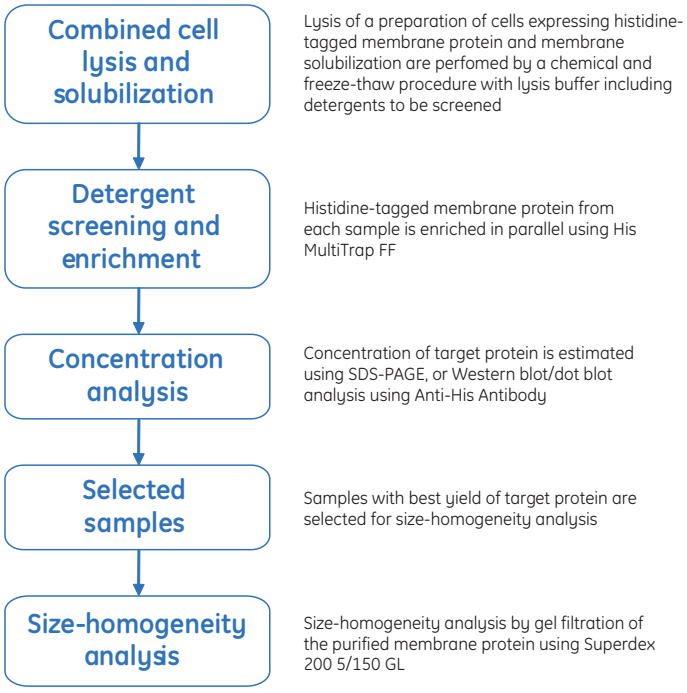

Fig 1. Summary of the detergent screening, membrane protein enrichment, and size-homogeneity analysis using a combination of His MultiTrap FF and Superdex 200 5/150 GL.

Cell lysis and membrane protein solubilization

Materials

Solubilization and cell lysis of *E. coli* are achieved by chemical and freeze-thaw lysis using the following buffer:

Lysis buffer: 20 mM sodium phosphate, 100 mM sodium chloride, 20 mM imidazole, 0.5 mM TCEP, 5 U/ml benzonase (nuclease), 1 mg/ml lysozyme, EDTA-free protease inhibitor cocktail, 1% to 2% detergent to be screened, pH 7.4

* The detergent to be screened is used in each sample, respectively.

Method

Use the following procedure for lysis and solubilization of the membrane protein:

1. Harvest cells from the culture by centrifugation at 8000 × g for 10 min or at 1000 to 1500 × g for 30 min at 4°C.
2. Discard the supernatant. Place the bacterial pellet on ice.
3. Suspend the bacterial pellet by adding 5 to 10 ml of lysis buffer for each gram of wet cells.
4. Leave for 2 h with mild agitation at room temperature or 4°C, depending on the sensitivity of the target protein.
5. Measure and adjust pH if required.

Enrichment of membrane protein

Materials

Sample: 100 µl unclarified cell lysate containing histidine-tagged membrane protein and detergent to be screened.

Detergents for screening: 1% Fos-Choline 12 (FC12); 1% undecyl maltoside (UDM); 1% dodecyl maltoside (DDM); 1% Cymal™5; 1% Cymal 6; 2% octyl glucoside (OG); 1% Triton™ X-100 (TX-100), 1% lauryl dimethylamine oxide (LDAO). All concentrations are chosen to be above the CMC for each detergent.

Binding buffer: 20 mM sodium phosphate, 500 mM sodium chloride, 20 mM imidazole, 0.5 mM TCEP, 1% to 2% detergent, pH 7.4

Wash buffer: 20 mM sodium phosphate, 500 mM sodium chloride, 40 mM imidazole, 0.5 mM TCEP, 0.03% dodecyl maltoside (DDM), 1% to 2% detergent, pH 7.4

Elution buffer: 20 mM sodium phosphate, 500 mM sodium chloride, 500 mM imidazole, 0.5 mM TCEP, 0.03% dodecyl maltoside (DDM), 1% to 2% detergent, pH 7.4.

Multiple, unclarified samples are enriched on His MultiTrap FF plate by centrifugation.

Method

1. Peel off the bottom seal of the His MultiTrap FF 96-well plate.
   Note: *Hold the plate above a sink due to risk of small leakage of storage solution when removing the bottom seal.*
2. While holding the plate in the upside down position, gently shake to dislodge any affinity medium stuck to the top seal.
   Note: *Each well contains 500 µl of a 10% slurry of Ni Sepharose™ Fast Flow in storage solution (50 µl medium in 20% ethanol).*
3. Place the plate in an upright position and peel off the top seal while holding the plate against the bench surface.
4. Position the plate on top of a 500 µl V-bottom Collection Plate, code number 28-4039-43
   Note: *Remember to change or empty the V-bottom Collection Plate when necessary during the following steps.*
5. Centrifuge the plates for 2 min at 500 × g, to remove storage solution from the medium.
6. Add 500 µl of deionized water/well and centrifuge for 2 min at 500 × g.

7. Add 500 µl of binding buffer/well and mix briefly, to equilibrate the medium. Centrifuge for 2 min at 500 × g. Repeat.
8. Apply 100 µl of sample to each well, mix briefly and incubate for 3 min. (increase the incubation time if the yield is too low).
9. Remove the flowthrough by centrifuging for 4 min at 100 × g (or until all wells are empty).
10. Add 500 µl binding buffer/well and mix briefly to wash out unbound sample. Centrifuge at 500 × g for 2 min.
11. Repeat once (or until all unbound sample is removed; detection at 280 nm [A280] should be < 0.1 for high purity).
12. Add 200 µl of elution buffer/well and mix for 1 min. Centrifuge the plates at 500 × g for 2 min and collect the fractions. Repeat twice (to give three eluates in total) or until all target protein has been removed. If required, change collection plate between each elution (to prevent unnecessary dilution of the target protein).

Further details on the use of this product are found in the Instructions for His MultiTrap FF (code no. 11-0036-62 AD).

Concentration analysis

The enrichment on His MultiTrap FF often makes it possible to use SDS-PAGE for determination of concentration of the target membrane protein. Analyze the first two elution fractions from the enrichment on His MultiTrap FF for each detergent screened (Fig 2). If a more sensitive and specific method is required, quantitative Western blot or dot blot analyses (Fig 3) using Anti-His Antibody (GE Healthcare code no. 27-4710-01) can be performed. For an overview of GE Healthcare ECL Western blotting products, see reference 1.

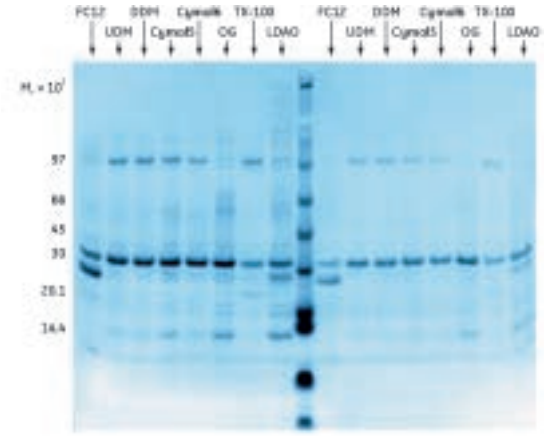

Fig 2. SDS-PAGE (Coomassie™ staining) of eluates 1 and 2 (lanes 1-8, and 10-17) from detergent screening with His MultiTrap FF of the membrane protein EM29. Molecular weight standard in lane 9. For full details, see reference 2.

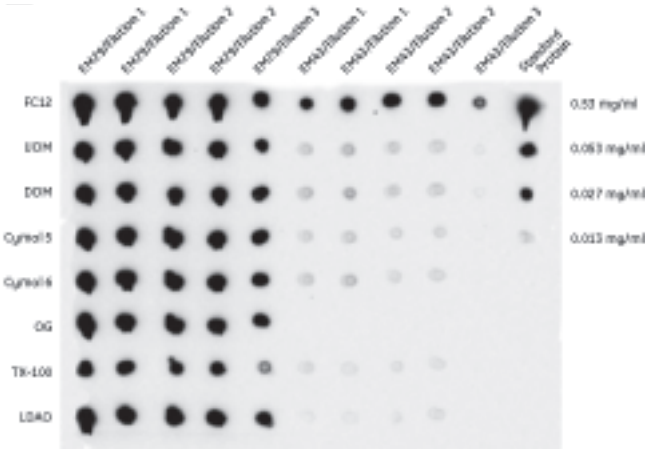

Fig 3. Dot-blots using nitrocellulose membrane followed by histidine tag-specific detection of membrane proteins purified on His MultiTrap FF. For full details, see reference 2.

Size-homogeneity analysis

Gel filtration is the method of choice for rapid detection of aggregation. The method can be applied under a wide variety of conditions. It is widely used as an efficient assay to assess the size homogeneity in purified membrane protein samples. Eluted fractions with acceptable yields of target membrane protein as determined by SDS-PAGE, Western blotting, or dot blotting in the concentration analysis are analyzed for size homogeneity by gel filtration on Superdex 200 5/150 GL. This column requires only 4 to 50 µl sample volumes, and only small volumes of detergent-containing eluent.

Materials

Sample: Eluted fractions from detergent screening step

Equilibration buffer: 20–50 mM sodium phosphate, 100 mM NaCl, X% detergent (optimal detergent for highest yield as determined from analysis of detergent screening step; concentration from 1.2- to 2-fold critical micellar concentration), pH 5–9

System: ÄKTAdesign™ system such as ÄKTAexplorer™

Method

1. Equilibrate the column using 6 ml equilibration buffer at a flow rate of 0.3 ml/min.
2 Apply 10 µl of eluent selected from the detergent screening step.
3. Continue elution with 4.5 ml equilibration buffer, measuring absorbance at 280 nm. Maintain pressure on the column below 1.5 MPa.

For detailed instructions on setting up the column for use on a chromatography system, see Instructions 18-1163-79 AD supplied with the product.

Comments

A single peak on the resulting chromatogram corresponds to a size-homogeneous protein and is often considered most suitable for crystallization. Additional peaks in the chromatogram are usually an indicator of undesirable multimeric or aggregated target membrane protein or contaminants. Figure 4 shows a typical gel filtration of an integral membrane protein (Mr 60 000) to screen for homogeneity under various pH and salt conditions. A symmetrical peak was obtained when the purification was performed at pH 5.2 in 0.1 M NaCl, indicating a homogenous protein under these conditions. At higher salt concentration (0.3 M), a small peak appeared close to the void volume, indicating that oligomerization or aggregation appeared to a limited extent. At both pH 7.5 and pH 9.5, significant peaks were obtained close to the void volume, indicating severe oligomerization or aggregation. The complete screening procedure was performed in less than 2 h, including the time for column equilibrations. Sample consumption was 6 × 10 µl for the complete screen.

For further details, see reference 3 and 4.

Column:	Superdex 200 5/150 GL
Sample:	Integral membrane protein (M_r 60 000) from *E. coli*
Sample volume:	10 µl
Eluents (including 0.1 or 0.3 M NaCl):	Buffer 1: 20 mM sodium acetate, 0.03 % dodecyl maltoside, 0.5 mM TCEP, pH 5.2; Buffer 2: 20 mM HEPES, 0.03 % dodecyl maltoside, 0.5 mM TCEP, pH 7.5 Buffer 3: 20 mM CAPSO, 0.03 % dodecyl maltoside, 0.5 mM TCEP, pH 9.5
Flow rate:	0.35 ml/min
Detection:	UV absorbance at 280 nm
System:	ÄKTAexplorer

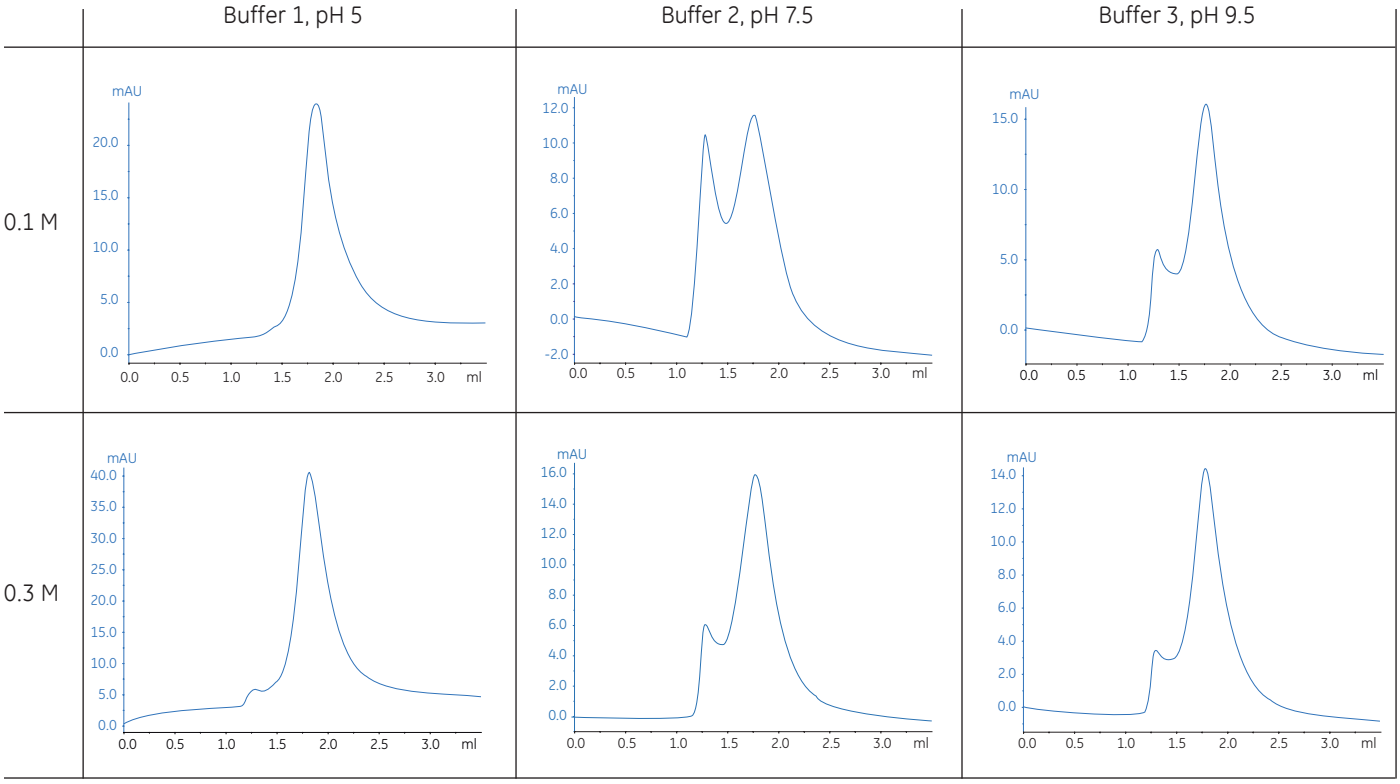

Fig 4. Typical chromatogram of detergent solubilized membrane proteins analyzed on Superdex 200 5/150 GL.

Purity analysis of membrane protein

Final purity of the membrane purity can be performed by SDS-PAGE.

References

1. GE Healthcare online, Western blotting: www.gelifesciences.com/ecl
2. Data file: His MultiTrap FF and His MultiTrap HP, GE Healthcare, 11-0036-63, Edition AB (2007).
3. *Purifying Challenging Proteins:* Principles and Methods, GE Healthcare 28-9095-31, Edition AA (2007), pp 15–30. http://www.gelifesciences.com/handbooks
4. *Purifying Challenging Proteins:* Principles and Methods, GE Healthcare 28-9095-31, Edition AA (2007), pp 40–42. http://www.gelifesciences.com/handbooks

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