Chapter 16

Detergent-Free Membrane Protein Purification

Alice J. Rothnie

Abstract

Membrane proteins are localized within a lipid bilayer; in order to purify them for functional and structural studies the first step must involve solubilizing or extracting the protein from these lipids. To date this has been achieved using detergents which disrupt the bilayer and bind to the protein in the transmembrane region. However finding conditions for optimal extraction, without destabilizing protein structure, is time consuming and expensive. Here we present a recently-developed method using a styrene-maleic acid (SMA) co-polymer instead of detergents. The SMA co-polymer extracts membrane proteins in a small disc of lipid bilayer which can be used for affinity chromatography purification, thus enabling the purification of membrane proteins while maintaining their native lipid bilayer environment.

Key words Membrane proteins, Solubilization, Purification, SMALP, Polymer, Nanodisc, Detergent

1 Introduction

Transmembrane proteins carry out a wide range of vital roles, including controlling what enters and leaves a cell and mediating intracellular communication. Consequently they are the target of a large number of prescribed drugs. However understanding exactly what they look like and how they work is more difficult than for soluble proteins due to their location within a membrane bilayer. To purify membrane proteins they must be extracted or solubilized from the lipid bilayer. To date this has been achieved using detergents/surfactants, which destabilize the membrane and interact with the membrane protein, creating a micellar structure around the regions that would normally be in the membrane. This approach has proven successful for many proteins, including all those for which an X-ray crystal structure has been obtained so far. However the use of detergents is not without difficulties, such as (1) striking the right balance between efficient extraction from the membrane without also denaturing the protein; (2) stripping away annular lipids from the protein which are crucial for function; and (3) loss of lateral pressure provided by the membrane which affects

Isabelle Mus-Veteau (ed.), *Heterologous Expression of Membrane Proteins: Methods and Protocols,* Methods in Molecular Biology, vol. 1432, DOI 10.1007/978-1-4939-3637-3_16,

both structure and function. Exactly which detergent will work best for a given protein cannot be easily predicted and therefore tends to involve a lengthy and expensive trial-and-error process, further complicated by the fact that the best detergents for extraction are not always the best for downstream applications. Recently we, and others, have demonstrated a new approach to membrane protein extraction/solubilization without the use of detergents, instead using a styrene-maleic acid (SMA) co-polymer [1–9]. The SMA co-polymer inserts into a biological membrane and forms small discs of bilayer encircled by the polymer (Fig. 1b) [10], which we term SMA lipid particles (SMALPs), but are also called lipodisqs [8, 9] or native nanodiscs [5]. SMALPs are small, soluble and stable, and proteins within SMALPs can easily be purified using affinity chromatography. SMALP-encapsulated proteins have been shown to be more thermostable than detergent-solubilized proteins [1, 3, 5]. The small-sized SMALPs do not significantly scatter light like proteoliposomes, and the polymer does not provide a large interfering signal, making them useful for various spectroscopic and biophysical techniques [1–4, 9, 11]. As both sides of the membrane are freely available they are ideal for membrane protein-binding assays [1, 3]. They have also been successfully utilized for structural studies using either negative stain or

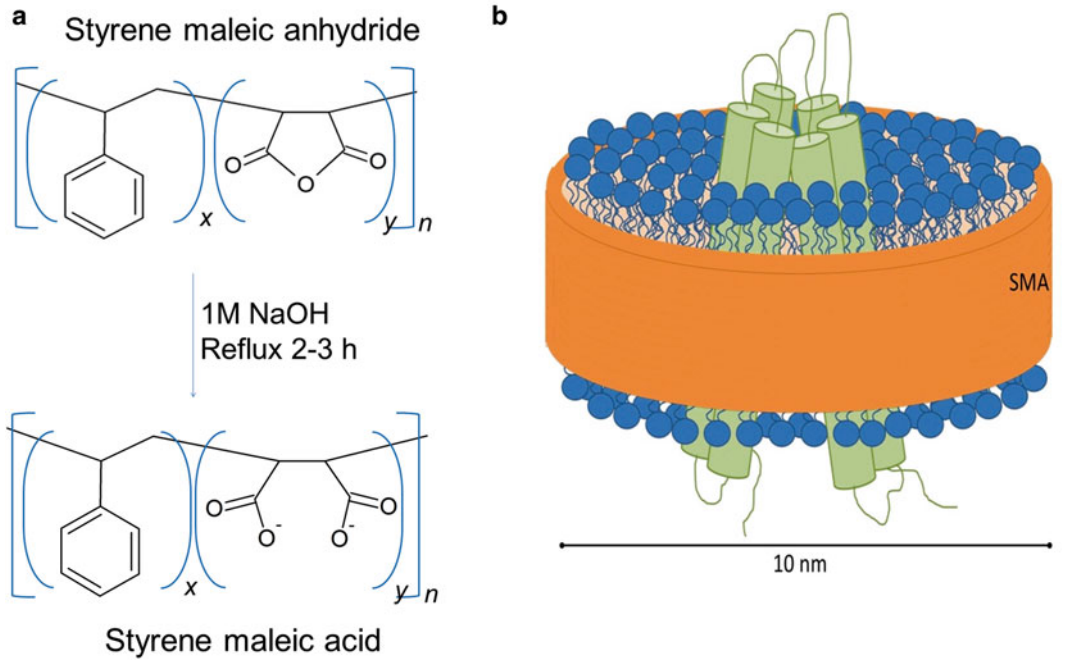

Fig. 1 SMA co-polymer and SMALP structures. (**a**) The SMA2000 polymer (Cray Valley) is a styrene-maleic anhydride co-polymer that must be hydrolyzed to form a styrene-maleic acid co-polymer. (**b**) The SMA co-polymer encircles a disc of lipid bilayer, effectively solubilizing the transmembrane protein while maintaining its lipid environment

cryo-electron microscopy [1, 7]. It is therefore possible to extract, purify and study the structure and function of a membrane protein while retaining its natural bilayer environment.

2 Materials

2.1 SMA Polymer Preparation

1. SMA2000 (styrene-maleic anhydride) powder: This polymer has a 2:1 ratio of styrene:maleic anhydride and a molecular weight of 7.5 kDa (Cray Valley).
2. 1 M NaOH solution.
3. Concentrated HCl (SG 1.18).
4. 0.6 M NaOH solution.
5. Distilled water.

2.2 Membrane Protein Extraction and Purification

1. Membrane preparations from cells expressing the target protein (*see* **Note 1**).
2. Buffer 1: 20 mM Tris–HCl pH 8, 150 mM NaCl, 10% (v/v) glycerol (*see* **Note 2**).
3. Ni^{2+}-NTA (Ni^{2+}-nitrilotriacetate) agarose resin, and an empty gravity flow column.
4. 2 M Imidazole.
5. Standard SDS-PAGE and Western blotting equipment and reagents.

3 Methods

3.1 SMA Co-polymer Preparation

SMA2000 is a styrene-maleic anhydride co-polymer. To be active for membrane solubilization it must be hydrolyzed to form styrene-maleic acid (Fig. 1a).

1. Dissolve 25 g SMA2000 powder in 250 ml 1 M NaOH overnight at room temperature using a magnetic stirrer and a round-bottomed flask (*see* **Note 3**).
2. In a fume hood put the round-bottomed flask containing the dissolved SMA2000 on a heating mantle and attach a condenser. Bring the solution to a boil and then reflux the polymer solution for 2–3 h. Allow to cool.
3. Divide the polymer solution between four 250 ml centrifuge tubes. In a fume hood gradually add concentrated HCl to each one, mixing well, to precipitate the polymer. Approximately 1 ml HCl per 6 ml polymer solution is required. Then add 100 ml distilled water to each tube and mix well.
4. Centrifuge at 10,000 × *g* for 10 min at room temperature and carefully pour off supernatant.

5. Add 150 ml distilled water to each tube and resuspend the polymer by shaking.
6. Centrifuge at 10,000 × *g* for 10 min at room temperature and carefully pour off supernatant.
7. Repeat **steps 5** and **6** four times.
8. Dissolve the polymer by adding 60 ml 0.6 M NaOH to each tube and either shaking or stirring for several hours.
9. Check the pH and adjust to pH 8.
10. Freeze-dry the SMA co-polymer.
11. Store at room temperature.

3.2 Membrane Protein Extraction and Purification

1. Resuspend the membrane preparation in buffer 1 at a concentration of 30 mg/ml wet weight of membrane pellet (*see* **Note 4**).
2. Add SMA co-polymer powder (from Subheading 3.1, **step 11**) to the resuspended membranes to give a final concentration of 2.5 % (w/v) (*see* **Notes 5** and **6**).
3. Incubate at room temperature for 1 h, shaking (*see* **Notes 7** and **8**).
4. Centrifuge at 100,000 × *g* for 20 min at 4 °C and harvest the supernatant containing the solubilized protein.
5. Measure solubilization efficiency by running a Western blot of solubilized sample against insoluble (resuspend the pellet in buffer 1 supplemented with 2 % (w/v) SDS) (Fig. 2a).
6. Mix the solubilized protein with Ni^{2+}-NTA resin (pre-washed in buffer 1), at a ratio of 100 µl resin/ml solubilized protein, and mix gently overnight at 4 °C (*see* **Notes 9** and **10**).
7. Pour into an empty gravity-flow column and wash the resin five times with 10 bed volumes (bv) of buffer 1 supplemented with 20 mM imidazole (*see* **Note 11**).
8. Wash resin twice with 10 bv of buffer 1 supplemented with 40 mM imidazole.
9. Wash once with 1 bv of buffer 1 supplemented with 60 mM imidazole.
10. Elute six times with ½ bv of buffer 1 supplemented with 200 mM imidazole.
11. Run samples from each step on SDS-PAGE and stain (either silver stain or Coomassie, depending on the abundance of the protein) (Fig. 2b).
12. Pool elution fractions containing the protein of interest, and remove any remaining free SMA by gel filtration and/or concentrate the sample using centrifugal concentrators (*see* **Note 12**).
13. Store sample for short term at 4 °C, or long term at −70 °C.

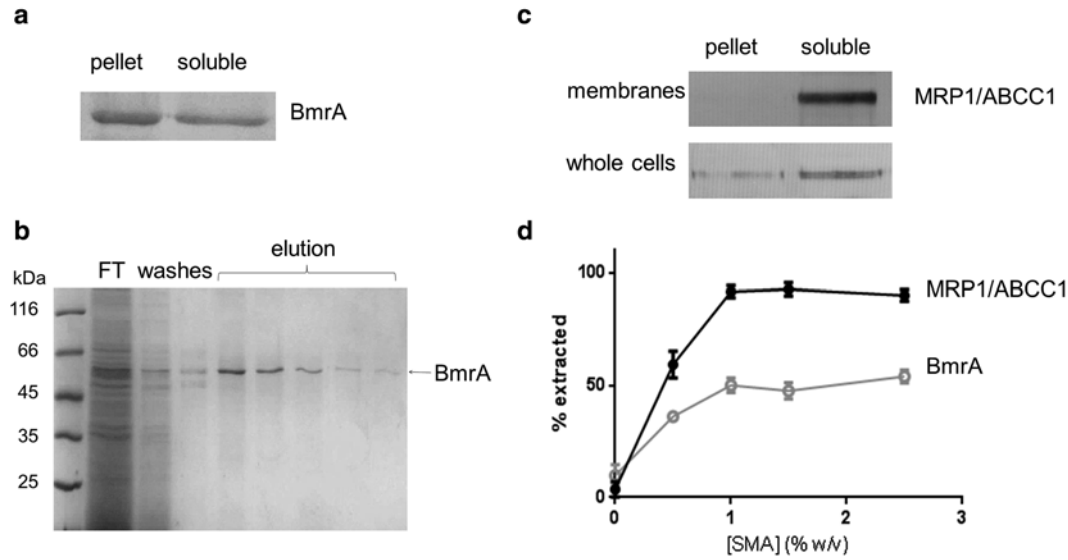

Fig. 2 Extraction and purification using SMA co-polymer. (**a**) Membrane preparations from C41 (DE3) E. coli overexpressing the ABC transporter BmrA were solubilized with 2.5 %(w/v) SMA for 1 h at room temperature, and then centrifuged at 100,000 g for 20 min. Samples of soluble and insoluble material were assayed by Western blotting using an anti-his primary antibody. (**b**) SMA-solubilized BmrA was purified using Ni^{2+}-NTA affinity chromatography. Samples of unbound protein, washes, and eluted protein were run on SDS-PAGE and stained with InstantBlue (Expedeon). (**c**) Human ABC transporter MRP1/ABCC1 overexpressed in H69AR cancer cells was extracted either from membrane preparations or whole cells. Soluble and insoluble fractions were analyzed by Western blot, using QCRL-1 as a primary antibody. (**d**) BmrA (open circles, grey) and MRP1 (closed circles, black) were solubilized with varying concentrations of SMA, and the % extracted (solubilized) analyzed by Western blot as in A & C, data are mean ± sem, $n \geq 3$

4 Notes

1. Membrane preparations are ideal, but whole cells can also be used (Fig. 2c) [5, 6], which will require addition of DNase. Membranes from all common expression systems (bacteria, insect cells, yeast, and mammalian cells [1] (Fig. 2)) work effectively. This protocol details the method for polyhistidine-tagged proteins, but other affinity chromatography methods can be used [1, 6].
2. The composition of this buffer is generally quite flexible, but the pH is important; a pH of 8 is ideal, certainly no lower than 7.5. Also divalent cations should be avoided. A low pH or divalent cations will prevent efficient extraction with the SMA co-polymer, or if added once the SMALP is formed, may cause it to precipitate.
3. Weigh out the SMA2000 in a fume hood. If some of the SMA2000 does not dissolve this does not matter. Always wear gloves when handling SMA since it can penetrate skin.

4. Many methods using detergents use a specific concentration of total membrane protein; however we use wet pellet weight of the membrane not protein concentration as it is the lipids the SMA co-polymer interacts with. To measure wet pellet weight, weigh an empty ultracentrifuge tube, then add your membrane, spin at 100,000 × *g* to pellet your membranes, carefully remove all of the supernatant, and weigh the tube again. The difference in weight from the empty tube gives you the wet pellet weight.
5. We simply add powder to the membrane suspension, because it is more convenient for long-term storage of the polymer, but alternatively a concentrated stock solution in buffer 1 can be prepared and mixed with the membrane suspension.
6. Although we use 2.5% (w/v) SMA co-polymer as a standard, successful solubilization can be achieved with lower concentrations (Fig. 2d).
7. Although the solution will noticeably appear clearer almost instantly, we have found that it often takes longer to achieve a good extraction of your membrane protein of interest. While 1 h at room temperature appears sufficient for most proteins we have tested [1], some proteins may require longer [3, 7].
8. The temperature is important because of the phase transition of the lipids. Since the SMA co-polymer interacts with the lipids rather than the protein it is important the temperature is above the phase-transition temperature so that the lipids are in the liquid phase [12]. While this may worry many membrane protein researchers who are used to maintaining everything at 4 °C during detergent solubilization, we have not found this to be a problem, presumably because the SMALP maintains stability of membrane proteins much better than detergents [1, 3]. In fact, for proteins that prove difficult to solubilize increasing the temperature to 37 °C for solubilization has been used [1, 3].
9. Binding of polyhistidine-tagged SMALP-encapsulated proteins to the Ni^{2+}-NTA resin is sometimes problematic. Possible reasons include interactions between the polyhistidine-tag and the co-polymer, steric hindrance from the co-polymer, or column spoiling by excess free SMA co-polymer. We have found that a dodeca-histidine tag is much better than a hexa-histidine tag for efficient binding. Sometimes increasing the concentration of NaCl in the buffer can improve binding, or decreasing the concentration of SMA co-polymer in the sample may help. We have also found that resins from different suppliers can affect binding efficiency in a protein-dependent manner; for example Ni^{2+}-NTA agarose (Qiagen) is better for some proteins tested, whereas HisPur Ni^{2+}-NTA (ThermoFisher) is better for others.
10. Rather than using Ni^{2+}-NTA resin and a gravity flow column, it is possible to use a HisTrap FF column (GE Healthcare) and the Akta system. In this case, load the column very slowly,

wash with buffer 1 until the A_{280} returns to baseline, and then elute using an imidazole gradient from 20 to 200 mM.

11. One of the biggest advantages of this method is that once formed the SMALPs are stable, and, unlike with detergent, it is not necessary to supplement the purification buffers or assay buffers with further SMA co-polymer.
12. Another big advantage of this method is that the protein can be concentrated easily using centrifugal concentrators. This is unlike detergent-solubilized proteins where problems are caused by simultaneously concentrating the detergent, protein is lost by sticking to the membranes of the concentrator, and the protein is prone to aggregation.

References

1. Gulati S, Jamshad M, Knowles TJ, Morrison KA, Downing R, Cant N, Collins R, Koenderink JB, Ford RC, Overduin M, Kerr ID, Dafforn TR, Rothnie AJ (2014) Detergent-free purification of ABC (ATP-binding-cassette) transporters. Biochem J 461:269–278
2. Knowles TJ, Finka R, Smith C, Lin YP, Dafforn T, Overduin M (2009) Membrane proteins solubilized intact in lipid containing nanoparticles bounded by styrene maleic acid copolymer. J Am Chem Soc 131:7484–7485
3. Jamshad M, Charlton J, Lin Y P, Routledge S J, Bawa Z, Knowles T J, Overduin M, Dekker N, Dafforn T R, Bill R M, Poyner D R, Wheatley M (2015) G-protein coupled receptor solubilization and purification for biophysical analysis and functional studies, in the total absence of detergent. Biosci Rep 35(2). pii: e00188
4. Swainsbury DJ, Scheidelaar S, van Grondelle R, Killian JA, Jones MR (2014) Bacterial reaction centers purified with styrene maleic acid copolymer retain native membrane functional properties and display enhanced stability. Angew Chem Int Ed Engl 53:11803–11807
5. Dorr JM, Koorengevel MC, Schafer M, Prokofyev AV, Scheidelaar S, van der Cruijsen EA, Dafforn TR, Baldus M, Killian JA (2014) Detergent-free isolation, characterization, and functional reconstitution of a tetrameric K^+ channel: the power of native nanodiscs. Proc Natl Acad Sci U S A 111:18607–18612
6. Paulin S, Jamshad M, Dafforn TR, Garcia-Lara J, Foster SJ, Galley NF, Roper DI, Rosado H, Taylor PW (2014) Surfactant-free purification of membrane protein complexes from bacteria: application to the staphylococcal penicillin-binding protein complex PBP2/PBP2a. Nanotechnology 25
7. Postis V, Rawson S, Mitchell JK, Lee SC, Parslow RA, Dafforn TR, Baldwin SA, Muench SP (2015) The use of SMALPs as a novel membrane protein scaffold for structure study by negative stain electron microscopy. Biochim Biophys Acta 1848:496–501
8. Long AR, O'Brien CC, Malhotra K, Schwall CT, Albert AD, Watts A, Alder NN (2013) A detergent-free strategy for the reconstitution of active enzyme complexes from native biological membranes into nanoscale discs. BMC Biotechnol 13:41
9. Orwick-Rydmark M, Lovett JE, Graziadei A, Lindholm L, Hicks MR, Watts A (2012) Detergent-free incorporation of a seven-transmembrane receptor protein into nanosized bilayer Lipodisq particles for functional and biophysical studies. Nano Lett 12:4687–4692
10. Jamshad M, Grimard V, Idini I, Knowles TJ, Dowle MR, Schofield N, Sridhar P, Lin YP, Finka R, Wheatley M, Thomas ORT, Palmer RE, Overduin M, Govaerts C, Ruysschaert JM, Edler KJ, Dafforn TR (2015) Structural analysis of a nanoparticle containing a lipid bilayer used for detergent-free extraction of membrane proteins. Nano Res 8:774–789
11. Sahu ID, McCarrick RM, Troxel KR, Zhang R, Smith HJ, Dunagan MM, Swartz MS, Rajan PV, Kroncke BM, Sanders CR, Lorigan GA (2013) DEER EPR measurements for membrane protein structures via bifunctional spin labels and lipodisq nanoparticles. Biochemistry 52:6627–6632
12. Scheidelaar S, Koorengevel MC, Pardo JD, Meeldijk JD, Breukink E, Killian JA (2015) Molecular model for the solubilization of membranes into nanodisks by styrene maleic Acid copolymers. Biophys J 108:279–290