Biological Systems: Open Access

Pramanik and Kumar, Biol Syst Open Access 2016, 5:2
http://dx.doi.org/10.4172/2329-6577.1000e114

Editorial BSO, an open access journal

Bottlenecks of Molecular Mass Determination of Oligomeric Membrane Protein - Detergent Complexes and What can be Achieved

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Received date: July 06, 2016; **Accepted date:** July 11, 2016; **Published date:** July 19, 2016

Introduction

Membrane proteins play a very fundamental role in the cellular processes and are often regarded as drug target. The hydrophobic nature of membrane proteins makes them challenging target for structural and functional characterization *in vitro* [1]. To make the membrane proteins amenable for in vitro work first of all they need to be extracted from biological membranes by treatment with mild detergents. Detergents solubilize membrane proteins by interacting with the hydrophobic part of the membrane proteins. Detergent molecules associate with the hydrophobic region of the membrane proteins and create a belt around the membrane proteins, which is necessary to keep them in solution and maintain the native or near native structure [2]. Therefore the extraction and purification process of membrane proteins yield a mixture of protein detergent complex and free detergent micelle. So any downstream process of further characterization of the membrane protein detergent complex (mPDC) is hindered by the presence of the unknown amount of detergent present in the preparation. The chemical and physical properties of different detergents used in membrane protein purification vary widely, making it difficult to build a common strategy to reduce the contribution of detergent fraction in mPDC [3]. Therefore it is nearly impossible to even deduce the molecular weight of the oligomeric membrane protein component from an mPDC.

To determine the molecular weight of the mPDC, most common laboratory methods employed are Blue Native PAGE [4-7] and gel filtration chromatography followed by light scattering [8-10], although mass spectrometry [11-13], analytical density gradient ultracentrifugation [14,15] and small angle X-ray scattering [16,17] may also be used. But none of these above mentioned methods can determine the definitive ratio between the protein component and detergent component in the mPDC. As noted, Blue Native PAGE is one of the most common methods to determine molecular weight of the oligomeric membrane protein complexes; there Coomassie blue dye replaces bound detergent from the mPDC of alpha helical inner membrane proteins. It has been shown with a plethora of membrane proteins that an average of 1.8 times gain in weight occurs to alpha helical membrane proteins due to Coomassie blue dye association during Blue Native PAGE [18]. Gel filtration chromatography followed by light scattering provides the hydrodynamic radius of the mPDC, which allows empirical determination of the molecular weight of the mPDC and not the protein component alone. To note, here density of detergent and protein component varies substantially, and each mPDC have different ratio of protein to detergent, henceforth the empirical studies are able to give at the best an approximate value for the molecular weight from their hydrodynamic radii. Mass spectrometry as such is not applicable for mPDCs, but a relatively new method of laser-induced liquid bead ion desorption mass spectrometry (LILBID-MS) allows detergent free molecular weight determination of molecular weight of oligomeric membrane proteins from mPDC as starting sample. In the process of LILBID-MS, the associated detergent molecules peel off from the oligomeric protein complex and the mass is determined for the naked protein component only. In analytical density gradient ultracentrifugation density of the molecule gets matched with the density of the carrier solution, but this density is for mPDC is an average of the contribution of protein and the detergent component and it is challenging to determine their relative fraction in the mPDC. Small angle X-ray scattering (SAXS) again give the hydrodynamic radii from which volumetric impression of the mPDC under study can be build upon but fails to determine the molecular weight of the protein component only.

To overcome this dilemma there need to be a way to quantitatively determine the detergent fraction in mPDC. Pramanik et al recently developed a simple way to do that when the detergent is having maltoside group. Detergents with maltoside head group carry carbohydrate moiety, which have been probed in their work to determine the amount of detergent present in mPDC [19]. Basically anthrone reagent was used which quantitatively measured the amount of maltoside headgroup present in the detergent, therby quantifying the detergent content as the maltoside head group is directly proportional to the amount of total detergent. Once the detergent fraction is known in mPDC, SAXS data, density gradient ultracentrifugation data or gel filtration data can then be reevaluated to know the molecular mass of the oligomeric protein complex without detergent contribution [19]. This method can be extended to any mPDCs as long as the detergent contains a carbohydrate head group. In anthrone method of carbohydrate determination, carbohydrates are dehydrated with concentrated H_2SO_4 to form furfural, which condenses with anthrone to form a green colored complex which can be measured colorimetrically at 620 nm by using a spectrophotometer. Above all anthrone method does not get interference from proteins or amino acids.

Similarly LILBID-MS is also successful in determining the molecular mass of protein component out of mPDC as starter sample. In LILBID-MS protein solutions were produced by a piezo driven droplet generator and introduced into vacuum via differential pumping. Therein the droplets were irradiated one by one by synchronized high-power mid-infrared laser pulses. The generated, irradiated droplets are then disrupted, resulting in the emission of ions from liquid into gas phase where they are mass analyzed in a time-of-

flight (TOF) reflectron mass spectrometer. LILBID-MS have been applied to many mPDCs to determine their oligomeric molecular mass. This has been very helpful to determine membrane protein oligomerization stoichiometry [20].

References

1. Charvat A, Abel B (2007) How to make big molecules fly out of liquid water: applications, features and physics of laser assisted liquid phase dispersion mass spectrometry. Phys Chem Chem Phys 9: 3335-3360.
2. Le Maire M, Champeil P, Moller JV (2000) Interaction of membrane proteins and lipids with solubilizing detergents. Biochim Biophys Acta 1508: 86-111.
3. Linke D (2014) Explanatory chapter: choosing the right detergent. Methods Enzymol 541: 141-148.
4. Dresler J, Klimentova J, Stulik J (2011) Bacterial protein complexes investigation using blue native PAGE. Microbiol Res 166: 47-62.
5. Glew MD, Veith PD, Chen D, Seers CA, Chen YY, et al. (2014) Blue native-PAGE analysis of membrane protein complexes in Porphyromonas gingivalis. J Proteomics 110: 72-92.
6. Schagger H (1995) Quantification of oxidative phosphorylation enzymes after blue native electrophoresis and two-dimensional resolution: Normal complex I protein amounts in Parkinson's disease conflict with reduced catalytic activities. Electrophoresis 16: 763-770.
7. Schagger H, Cramer WA, von Jagow G (1994) Analysis of molecular masses and oligomeric states of protein complexes by blue native electrophoresis and isolation of membrane protein complexes by two-dimensional native electrophoresis. Anal Biochem 217: 220-230.
8. Strop P, Brunger AT (2005) Refractive index-based determination of detergent concentration and its application to the study of membrane proteins. Protein Sci 14: 2207-2211.
9. Wen J, Arakawa T, Philo JS (1996) Size-exclusion chromatography with on-line light-scattering, absorbance and refractive index detectors for studying proteins and their interactions. Anal Biochem 240: 155-166.
10. Zouni A, Kern J, Frank J, Hellweg T, Behlke J, et al. (2005) Size determination of cyanobacterial and higher plant photosystem II by gel permeation chromatography, light scattering and ultracentrifugation. Biochemistry 44: 4572-4581.
11. Hoffmann J, Aslimovska L, Bamann C, Glaubitz C, Bamberg E, et al. (2010) Studying the stoichiometries of membrane proteins by mass spectrometry: Microbial rhodopsins and a potassium ion channel. Phys Chem Chem Phys 12: 3480-3485.
12. Hoffmann J, Sokolova L, Preiss L, Hicks DB, Krulwich TA, et al. (2010) ATP synthases: Cellular nanomotors characterized by LILBID mass spectrometry. Phys Chem Chem Phys 12: 13375-13382.
13. Mager F, Sokolova L, Lintzel J, Brutschy B, Nussberger S (2010) LILBID-mass spectrometry of the mitochondrial preprotein translocase TOM. J Phys Condens Matter 22: 454132.
14. Le Roy A, Wang K, Schaack B, Schuck P, Breyton C, et al. (2015) AUC and small-angle scattering for membrane proteins. Methods Enzymol 562: 257-286.
15. Salvay AG, Santamaria M, le Maire M, Ebel C (2007) Analytical ultracentrifugation sedimentation velocity for the characterization of detergent-solubilized membrane proteins Ca++-ATPase and ExbB. J Biol Phys 33: 399-419.
16. Chen PC, Hub JS (2015) Structural properties of protein-detergent complexes from SAXS and MD simulations. J Phys Chem Lett 6: 5116-5121.
17. O'Neill H, Heller WT, Helton KE, Urban VS, Greenbaum E (2007) Small-angle X-ray scattering study of photosystem I-detergent complexes: implications for membrane protein crystallization. J Phys Chem B 111: 4211-4219.
18. Heuberger EH, Veenhoff LM, Duurkens RH, Friesen RH, Poolman B (2002) Oligomeric state of membrane transport proteins analyzed with blue native electrophoresis and analytical ultracentrifugation. J Mol Biol 317: 591-600.
19. Pramanik A, Zhang F, Schwarz H, Schreiber F, Braun V (2010) ExbB protein in the cytoplasmic membrane of *Escherichia coli* forms a stable oligomer. Biochemistry 49: 8721-8728.
20. Pramanik A, Hauf W, Hoffmann J, Cernescu M, Brutschy B, et al. (2011) Oligomeric structure of ExbB and ExbB-ExbD isolated from *Escherichia coli* as revealed by LILBID mass spectrometry. Biochemistry 50: 8950-8956.