Size Exclusion Phases for Membrane Protein Separations

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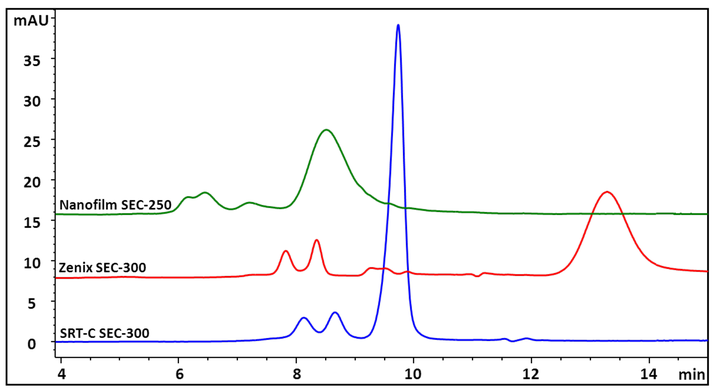
INTRODUCTION

Membrane proteins are a major part of pharmaceutical targets for disease therapeutics. Membrane protein's characterization and structural biology are bottlenecks in biomedical research due to their low abundance in cell lysates and the difficulty in their isolation. Their large sizes and hydrophobic nature pose great challenges in membrane protein separations. Here we present a set of size exclusion tools to find the best surface chemistry for different proteins with varying hydrophobicity. The size exclusion phases used include three different chromatographic surfaces: Zenix™ SEC-300, Zenix™-C SEC-300 and Nanofilm™ SEC-250. Both Zenix™ and Zenix™-C SEC-300 are made of 3 µm particles with 300 Å pore size, while SRT-C SEC-300 and Nanofilm™ SEC-250 are made of 5 µm particles with 300 and 250 Å pore sizes, respectively. Zenix™ SEC-300 has a stand-up monolayer bonded onto porous silica particles. Zenix™-C SEC-300 and SRT-C SEC-300 both have a lay-down monolayer bonded onto porous silica particles. Nanofilm™ SEC-250 is made of nanometer thick polymer coatings. These size exclusion phases enable the selection of a better SEC surface chemistry for individual protein separations.

EXPERIMENTAL

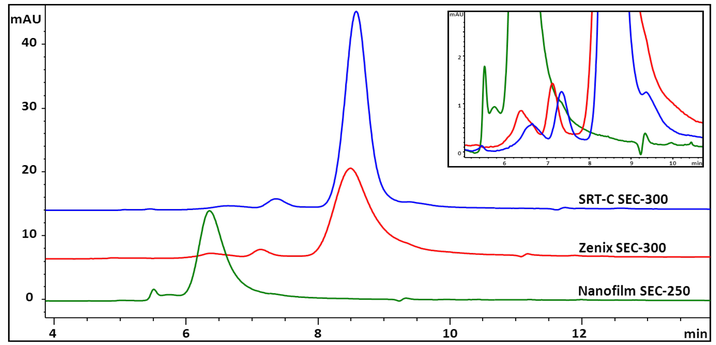
HPLC System: Agilent 1200 HPLC with binary pump
SEC columns: Zenix™ SEC-300 7.8x300mm
Zenix™-C SEC-300 7.8x300mm
Nanofilm™ SEC-250 7.8x300mm
SRT-C SEC-300 7.8x300mm
Mobile Phases: 20 mM Tris-HCl pH 7.0 with 190 mM NaCl,10 mM KCl, and 40 mM OG
10 mM Tris, pH 8.0 with 0.1% LDAO
Flow rate: 1.0 and 0.8 mL/min
Detection UV 214 nm
Column Temperature: 25 °C
Samples: Aqpz (6.0 mg/mL)
LeuT (1.0 mg/mL)
Rhodo bacteria Lysate
Purified Bacterial Photosynthetic Reaction Center (RC) Membrane Protein (1.0 mg/mL)

LeuT SEPARATION ON THREE SEC PHASES



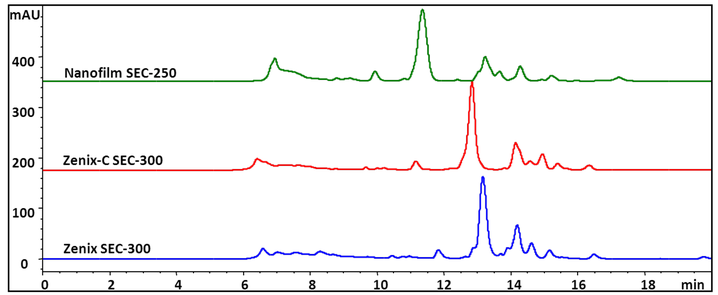
Analysis of the membrane protein LeuT on three different size exclusion phases. The mobile phase was 20 mM Tris-HCl pH 7.0 with 190 mM NaCl, 10 mM KCl, and 40 mM OG. The flow rate was 1.0 mL/min. 10 µL of the membrane protein (1.0 mg/mL concentration) was injected onto each column. Detection is at UV 280 nm.

Aqpz SEPARATION ON THREE SEC PHASES



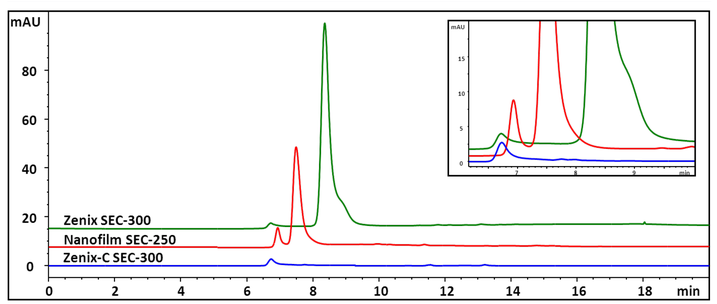
Analysis of the membrane protein Aqpz on three different size exclusion phases. The mobile phase was 20 mM Tris-HCl pH 7.0 with 190 mM NaCl, 10 mM KCl, and 40 mM OG. The flow rate was 1.0 mL/min. 2 µL of the membrane protein (6.0 mg/mL concentration) was injected onto each column. Detection is at UV 280 nm.

RHODO BACTERIA LYSATE ON THREE SEC PHASES



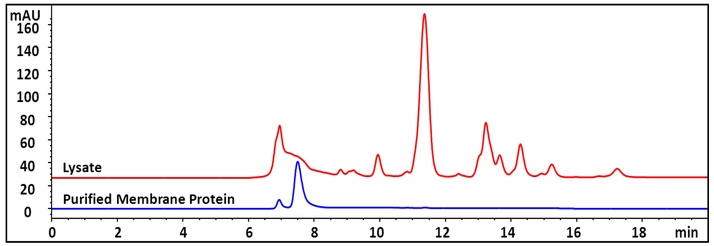
Analysis of rhodo bacteria lysate on three different size exclusion phases. The mobile phase was 10 mM Tris, pH 8.0 with 0.1% LDAO. The flow rate was 0.8 mL/min. 5 µL of the membrane protein was injected onto each column. Detection is at UV 280 nm.

PURIFIED RC MEMBRANE PROTEIN ON SEC PHASES



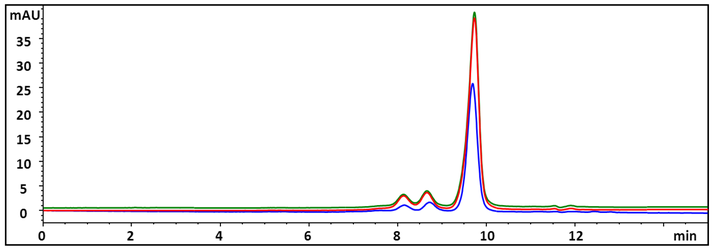
Analysis of purified bacterial photosynthetic reaction center (RC) membrane protein on three different size exclusion phases. The mobile phase was 10 mM Tris, pH 8.0 with 0.1% LDAO. The flow rate was 0.8 mL/min. 10 µL of the membrane protein (1.0 mg/mL concentration) was injected onto each column. Detection is at UV 280 nm.

ANALYSIS OF BACTERIA LYSATE AND PURIFIED RC MEMBRANE PROTEIN ON NANOFILM SEC-250



Analysis of rhodo bacteria lysate and a purified RC membrane protein on Nanofilm SEC-250. The mobile phase was 10 mM Tris, pH 8.0 with 0.1% LDAO. The flow rate was 0.8 mL/min. 5 µL of the Lysate and 10 µL of the purified membrane protein (1.0 mg/mL) was injected onto each column. Detection is at UV 280 nm.

STABILITY OF LeuT ON SRT-C SEC-300



Injections of LeuT on SRT-C SEC-300 over the course of one month. The mobile phase was 20 mM Tris-HCl pH 7.0 with 190 mM NaCl, 10 mM KCl, and 40 mM OG. The flow rate was 1.0 mL/min. 10 µL of LeuT (1.0 mg/mL and blue trace is 0.5 mg/mL) was injected onto each column. Detection is at UV 280 nm.

CONCLUSION

- With well-designed surface coating properties, these SEC packings can selectively elute membrane proteins based on their structure and hydrophobicity.
- SRT-C SEC-300 gave the best separation of LeuT with the least hydrophobic interaction and the best separation of Aqpz with less secondary interactions; baseline separation with the high molecular weight proteins.
- Nanofilm SEC-250 showed the best performance for the separation for the analysis of purified bacterial photosynthetic reaction center (RC) membrane protein.
- The SRT-C SEC-300 column is stable in a mobile phase containing OG detergent; the column showed no change in peak shape or retention time over multiple injections and after long term column storage in detergent.

REFERENCES

1. Stabilization of the Human B2-Adrenergic Receptor TM4–TM3–TM5 Helix Interface by Mutagenesis of Glu1223.41, A Critical Residue in GPCR Structure, J. Mol. Biol. 376, 1305-1319 (2008) β

2. The Bacterial Photosynthetic Reaction Center as a Model for Membrane Proteins, Annu. Rev. Biochem., 58: 607-33 (1989)

Acknowledgements to Brad C. Bennett from the University of Virginia and Kelley Kearns from the University of Delaware.