Utilization of HPLC-ELSD for the Separation and Detection of Detergents and Lipids in Proteoliposomes Samples Prepared for ssNMR Experiments

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Introduction

The three dimensional structure of any given protein is determined by the amino acid sequence in a given environment.[1] Therefore, a native-like environment should be used when investigating proteins. For membrane proteins, the native environment is a membrane. Unfortunately sample preparations for membrane proteins in lipid bilayers typically require the use of detergents to initially solubilize the protein. Once the protein has been mixed with lipids the detergent can be removed through either dialysis or through the use of a chemical such as cyclodextran.[2] However, the ease of removal of detergent from the proteoliposomes varies depending on the protein and lipids. Therefore, the ability to separate the components of the sample and detect trace detergent would be useful. Because most lipids and detergents lack a chromophore common types of detection, such as UV, cannot be used. Evaporative light scattering detection (ELSD) relies on a desolvated analyte scattering light. The signal intensity is dependent on the diameter of the dry analyte, which allows for quantitative analysis. The HPLC-ELSD method was used to confirm the removal of detergents from proteoliposomes, to develop sample preparation methods for several membrane proteins that will be studied using ssNMR, and to minimize dialysis time for the membrane protein Rv1861.

Experimental

The membrane protein Rv1861 from *Mycobacterium tuberculosis* (TB), is over-expressed in *E. coli*, purified via Ni-affinity chromatography, solubilized in the detergent DDGly, and reconstituted into DMPC/DMPG (4:1) proteoliposomes. After each daily dialysis buffer change, an aliquot of the detergent-proteoliposome mixture was removed from the dialysis bag. Subsequently, HPLC analysis utilizing an ELSD system (Agilent Technologies, ELSD 380) was used to monitor the concentration of detergent. The aliquots were not diluted prior to each 25µL injection. An Acclaim™ Surfactant Plus 3.0µm (3.0 X150 mm) analytical HPLC column was used for the separation. An AB linear gradient elution at 30°C from 70% to 15% eluent B over 10 min. at a flow rate of either 0.8 or 0.9 mL/min was used. Eluent A was HPLC grade acetonitrile and eluent B was 0.1M Ammonium Acetate at pH 5.0 for the separation.

Results and Discussion

Utilizing HPLC-ELSD, common detergents were separated, all with baseline resolution as shown in Figure 1. The inset of Figure 1 shows a peak corresponding to a pM DDGly, demonstrating the limit of detection for the Agilent ELS detector. At picomolar concentrations, there would be less than one molecule of detergent per ten thousand molecules of protein. Thus, clear resolution of molecules with similar chemical structure can be obtained utilizing this analytical HPLC column; and the limits of detection are sensitive enough to ensure that samples prepared for ssNMR have negligible residual detergent.

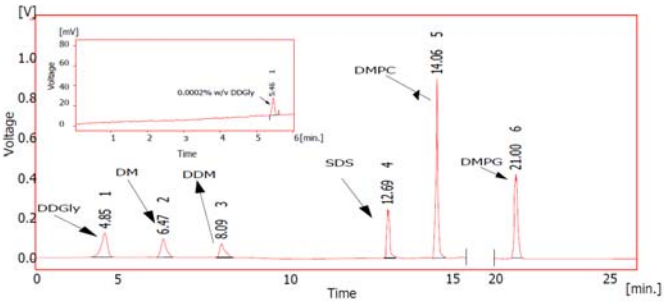

Figure 1. HPLC-ELSD chromatogram showing the separation of several detergents and lipids.

The HPLC-ELSD technique has the additional benefit of a clear separation in retention times between lipids and detergents. All synthetic lipids, and most of the native *E. coli* lipids, have retention times greater than fourteen minutes. Detergents however, have retention times of less than fourteen minutes. And to date, the described method has been used on samples containing the TB proteins Rv1861, CrgA, FtsX with varying lipids and detergent. This indicates that the developed technique is robust enough to easily transfer the method to new systems.

Acknowledgements

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References

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[2] Das, N., *et al.,* Nature Protocols **8**, 2256–2270 (2013).