Elucidating Algal Extracellular Polymeric Substance Structures with Asymmetrical Flow Field-Flow Fractionation and Light Scattering

Kaitlin Lesco1,2, Christine Plavchak1, S. Kim R. Williams1, Lieve M. L. Laurens 2

1Department of Chemistry, Colorado School of Mines, Golden, CO 80401, 2 The National Renewable Energy Laboratory, Golden, CO, 80401

Introduction

Extracellular polymeric substances (EPS) from algae are complex, secreted, aquatic heteropolymers, possibly functioning as carbon sinks. EPS have tremendous potential to be utilized as high-value coproducts, e.g., hydrocolloids or bio-based polymers, and play a significant role in the overall aquatic ecology (feeding a healthy microbiome) during cultivation. Unfortunately, the structural elucidation of these polymers is elusive making the design of custom applications difficult. We must characterize these polymers on a chemical, structural, and physical level to understand their biological significance and industrial potential. The first step is to reduce the complexity of EPS with a size-based separation such as asymmetrical flow field-flow fractionation (AF4). When AF4 is coupled to multi-angle light scattering (MALS), the molecular weight and size of different populations in the sample can be measured. This work evaluates the different size populations present in the EPS of Chlorella vulgaris using AF4-MALS.

Basic Principles of AF4

Step 1. Sample is injected into channel
Step 2. Analytes are focused and concentrated into a thin band using opposing carrier fluid flow
Step 3. Perpendicular cross flow concentrates analytes above accumulation wall. This concentration is counteracted by diffusion of analytes away from accumulation wall
Step 4. Analytes reside in different flow velocity zones depending on their Brownian motion resulting in sized-base separation

Extracellular Polymeric Substances Chemistry

Extracellular polymeric substances are composed mainly of polysaccharides and proteins with minor components made of lipids and nucleic acids.1 Bulk compositional analysis has revealed that the polymer is 38% carbohydrates. Bulk zeta potential measurements revealed an anionic surface chemistry.

Initial batch DLS measurements distinguished two different size populations in the EPS of C. vulgaris. Using AF4 with online MALS, dRI and UV detectors we were able to establish molecular weight ranges for the different sizes. Differences in peak shape between the dRI and UV suggest heterogeneity in protein distribution between the polymers. Fraction 1 and fractions 2 and 3 (together) were collected for further compositional analysis.

The EPS of C. vulgaris is composed of different size distributions.

Table 1. Molecular weight ranges for C. vulgaris EPS in NaNO3

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Molecular Weight Range (Da)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50-50k</td>
</tr>
<tr>
<td>2</td>
<td>5-100M</td>
</tr>
<tr>
<td>3</td>
<td>100-500M</td>
</tr>
</tbody>
</table>

Different ionic strengths of carrier fluid were tested using NaNO3. Concentrations of 10, 20, 50, 75 and 100 mM were investigated. A consistent finding was that at high ionic strength fractions 2 and 3 aggregate and elute as one fraction. This is likely related to the salts screening the charges on the polymers and allowing for collapse of Debeye screening length resulting in aggregation.

Influence of Ionic Strength on EPS Quaternary Structure

The change in salt composition led to dramatically different elution profiles. Molecular weight ranges did not change drastically, however, based on concentration detectors, EPS in NaCl had a larger concentration of polymers with molecular weight ranging from 50M-100M whereas EPS in NaNO3 had a larger concentration of polymers ranging from 100M-500M indicating these high molecular weight polymers could be forming reversible aggregates that are both salt and ionic strength dependent.

Effect of Carrier Fluid Salt on EPS Retention and Aggregation

Salt composition of carrier fluid induces different aggregation patterns in the EPS from C. vulgaris.

Impact and Future Work

• AF4 is a capable technique for characterizing the quaternary structure of extracellular polymeric substances and allows us to not only characterize the different molecular weight populations but also the aggregation behavior in different carrier liquids.
• Differences in elution profiles are indicative of charge heterogeneity which could have large implications on alkalinity in the polymer’s native environment.
• Understanding both the structure and behavior of EPS will allow for deliberate utilization of the polymers in applications ranging from wastewater mitigation to food and nutraceuticals to carbon sequestration.
• Future works include obtaining carbohydrate data for specific polymeric fractions, testing MgCl2 as a carrier fluid and investigating the charge distribution and polymer architecture using mass spectrometry.

References


Acknowledgements

Financially supported through National Renewable Energy Laboratory AF4P with Colorado School of Mines, Department of Chemistry. For more information contact: lwla@mines.edu, or llaurens@nrel.gov